# Developing a strategy to improve the environmental risk assessment of substances of unknown and variable composition (MCS/ UVCB)

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*Total number of authors:*
11

*Published in:*
Society of Environmental Toxicology and Chemistry North America 39th Annual Meeting - abstract book

*Publication date:*
2018

*Document Version*
Publisher's PDF, also known as Version of record

# Sacramento

**Society of Environmental Toxicology and Chemistry**
**North America 39th Annual Meeting**

4–8 November 2018 | Sacramento, California

Bridging Divides Between Environmental Stewardship and Economic Development

# Abstract Book

SETAC North America 39th Annual Meeting

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This book comprises the abstracts of the presentations for the platform and poster sessions of the Society of Environmental Toxicology and Chemistry (SETAC) North America 39th Annual Meeting, conducted at the Sacramento Convention Center from 4–8 November 2018 in Sacramento, California. The abstracts are reproduced as accepted by the Scientific Program Committee and appear in numerical order.

In each abstract, the presenting author's name is underlined. The author index cross-references the corresponding abstract numbers. Affiliation, session and keyword indices are also included.

International Standard Serial Number 1087-8939

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- Promote research, education and training in the environmental sciences
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- Participate in the scientific interpretation of issues concerned with hazard assessment and risk analysis
- Support the development of ecologically acceptable practices and principles
- Provide a forum (meetings and publications) for communication among professionals in government, business, academia and other segments of society involved in the use, protection and management of our environment

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# Fate of Organic Contaminants in Aquatic Invertebrates and Their Environments – Measurement, Modeling and Risk Management

**1 Using benthic invertebrate life history data to improve bioaccumulation predictions for a contaminated Northeast estuary**

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Benthic invertebrates as a group have incredibly diverse forms and life histories, but they are often treated as a homogenous accumulator of sediment contamination. Given the relative importance of benthic invertebrates as a vector for chemical transfer to higher trophic levels, it may be beneficial to more accurately characterize benthic invertebrates when modeling bioaccumulation. Life history information, such as diverse feeding strategies and expected depth of sediment exposure (i.e., feeding depth), was incorporated when developing a food web model for a Northeast estuary. This better characterization of benthic invertebrates has improved model calibration and confidence in predicted fish tissue concentrations. Although regional data was used for this study, the collection of site-specific data is recommended for future work to reduce uncertainty and further improve the model.

**2 Modeling Bioconcentration of Organic Pollutants in Amphipods and Midges**

*D. Kuo, City University of Hong Kong / Architecture and Civil Engineering; C.C. Chen, City University of Hong Kong*

Amphipods and midges are important players in aquatic ecosystems. They have been widely used as test organisms in the ecotoxicological studies of potential contaminants including bioaccumulation potentials. With increasing shift towards non-sentient organisms as focus of experimental studies and the need for shorter experiments, amphipods and midges are ideal laboratory invertebrates for ecotoxicological studies and risk assessment. This study aims at developing bioconcentration models for amphipods and midges based on the toxicokinetics of uptake and removal processes and polyparameter-based linear free energy relationships (pp-LFERs). Bioconcentration and toxicokinetic data were gathered from primary bioaccumulation literature and reviewed following a 7-stage data quality assessment protocol. A total of 45 and 161 bioconcentration factors (BCFs) were collected for 6 amphipod and 21 midge species, respectively, covering 83 organic compounds with log $K_{OW}$ ranging from 0.57 to 7.62. The resulting BCF models generally performed well, with RMSE of 0.68 and 0.67 for amphipod and midge, respectively. For short-duration uptake experiments (i.e., £ 7d), BCFs measured from radioactivity analysis appear consistent with those derived with analysis of parent chemicals. The limitations of the developed models point to three immediate needs: build up high-quality experimental $k_M$ values for representative invertebrate species, explore the potential role of exoskeleton in chemical binding, examine the bioaccumulation of ionizable organic contaminants, and standardize experimental procedure for different invertebrates.

**3 What if Bioaccumulation is Concentration Dependent? A Meta-Analysis on Invertebrates Data**

*D. Kuo, City University of Hong Kong / Architecture and Civil Engineering*

Currently, bioaccumulation potential is measured and expressed with different bioaccumulation metrics such as bioconcentration factor (BCF) and biota-soil/sediment accumulation factor (BSAF). The underlying key assumption of these factors is that bioaccumulation does not vary with exposure concentration or chemical activity. Standard bioaccumulation experiments are often conducted in laboratory at concentrations that are analytically convenient rather than environmentally realistic, with the laboratory exposure levels typically higher than field conditions. It is thus critical that the concentration independent assumption is valid. This study examine this assumption by conducting a meta-analysis of bioaccumulation data of organic pollutants in invertebrates across different exposure media including soil, sediment, and aqueous solution. Over 100 chemical-medium cases were found and retrieved from primary experimental studies. Existing evidence suggest that standard bioaccumulation factors tend to decrease with increasing exposure concentration for both polar and hydrophobic organic compounds. If the observed concentration trend is generally true, laboratory generated bioaccumulation factors may be underestimating the bioaccumulation potential of chemicals. Furthermore, new approach to describe bioaccumulation measurements may be needed. A number of plausible causes and reasons for the dependence are examined and discussed.

**4 Persistent organic pollutant monitoring in the Anacostia watershed using passive sampling and uptake in a freshwater mussel**

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Located within a major urban environment, the Anacostia River continues to receive inputs of legacy pollutants from its watershed, resulting in accumulation in fish and the issuance of fish consumption advisories. Ongoing Remedial Investigation/Feasibility Study of the tidal Anacostia is aimed at identifying the extent and magnitude of contamination, assessing risks, and evaluating cleanup alternatives. Model predictions are also required to determine the concentration reductions in sediment needed to achieve target fish tissue concentration for the protection of human health. To address this aim, polychlorinated biphenyls (PCBs), polynuclear aromatic hydrocarbon (PAHs), and organochlorine (OC) pesticide concentrations in the water column and sediment porewater were monitored at several locations in the Anacostia River and major tributaries. A passive sampling approach was used and results were compared to quantify the dominant sources of pollution into the river. Caged mussels were concurrently deployed at the same tributary locations to measure in situ uptake in the tissue and determine if water concentrations are a good predictor for bioaccumulation at the lower trophic level of the food chain. Several organic pollutants in the Anacostia River water exceeded the EPA water quality criteria for the protection of human health. Our measurements indicate that some tributaries continue to serve as a source of the pollutants to the Anacostia River. Pollutant uptake was observed in mussel tissue over the three-month deployment period, in correlation with detected water concentrations. Partitioning-based bioconcentration models were tested and showed reasonable agreement within a factor of 2 to 10 between predicted and measured concentrations depending on the model tested. The overall results confirm that freely dissolved concentrations of persistent organic pollutants in the water column can be used to predict biouptake in filter-feeding benthic organisms. Results from this study are helping in the determination of the potential sources of contamination from tributaries into the River and developing better prediction of uptake in the aquatic food chain.

**5 Differential accumulation of select pharmaceuticals and contaminants of emerging concern in an effluent-dependent experimental stream mesocosm**

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Pharmaceuticals from wastewater treatment plant effluent are known to bioaccumulate in fish exposed to instream flows from urban ecosystems, particularly in arid and semi-arid regions; invertebrate bioaccumulation of these compounds is less defined. However, bivalves have been recently reported to accumulate some of the highest levels of antidepressants. For example, fish from an effluent dependent river accumulated up to 14 ± 4.6 µg/kg of the antidepressant sertraline compared to unionid mussels that accumulated sertraline to levels an order of magnitude higher (140±21 µg/

kg). Bioaccumulation differences among fish and bivalves have not been adequately studied and potentially result from different routes of exposure and disposition. *Corbicula fluminea*, an invasive freshwater bivalve, exhibits characteristics analogous to model organisms used for biomonitoring studies (e.g., broad distribution, rapid growth rate, high fecundity, ease of collection and transport, short acclimation periods). The goal of this study was to examine bioaccumulation and uptake of select target analytes by freshwater clams (*C. fluminea*), grazing fish (*Campostoma anomalum*), and periphyton during a replicated outdoor stream mesocosm study at the Baylor Experimental Aquatic Research facility. Municipal effluent, which was supplied from the Waco Metropolitan Area Regional Sewerage System, served as the stream flow source water. Organisms were sampled on study days 0, 1, 3, and 8. Target analytes were determined in whole tissue homogenates, source effluent and stream water by isotope dilution LC/MSMS. Following the 8-day study period, several target analytes were differentially accumulated between periphyton, fish and clams. For example, *C. fluminea* rapidly accumulated acetaminophen, carbamazepine, diphenhydramine, fluoxetine, and sertraline with maximum concentrations reaching low µg/kg levels. However, observed increases by study day 8 corresponded to increased target analyte concentrations in effluent source waters. Bioaccumulation dynamics of ionizable pharmaceuticals in *C. fluminea* and other bivalves (e.g., Unionids, estuarine and marine bivalves) requires additional study.

**6 Trait-based exposure assessment of Persistent Organic Pollutants in Antarctic Benthic Invertebrates**

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Persistent Organic Pollutants (POPs) can be found in invertebrates inhabiting ecosystems all around the world, even in Antarctica. However, research on Antarctic marine benthic organisms is scarce due to difficulties in collection and analysis of samples. In this study we perform for the first time an exhaustive survey of levels of POPs in different species of marine invertebrates of the Western Antarctic Peninsula (WAP) with different feeding behavior. Multiple (n>10) samples of sea squirt (*Cnemidocarpa verrucosa),* limpet *(Nacella concinna),* sea urchin *(Sterechinus neumayeri),* sea cucumber *(Heterocucummis steineni) and* sea star *(Odontaster validus)* were collected by divers in Ryder Bay near the British Rothera research station during the austral summer of 2016/2017. Samples originated from 2 locations: in the direct vicinity of the station and further away in the bay, allowing the quantification of the potential release of POPs, related to local human activities. The presentation will contain detailed results on polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and Polybrominated diphenyl ethers (PBDEs). Preliminary outcomes indicate distinctive patterns between species and different POPs. Limpets appear to have higher concentrations than other species, contradicting the hypothesis that predatory species, like the sea star *(Odontaster validus),* contain highest concentrations of these compounds. In comparison to other studies (exclusively conducted in Eastern Antarctica), concentrations of PCBs (ΣPCBs 2-15 ng/g lipid weight) are relatively (10-1000 times) low. This might result from the unique atmospheric and hydrological characteristics of the WAP. Among the OCPs, legacy pesticides like ones of the DDT and chlordane groups were still detectable in the Antarctic invertebrates, but at relatively low concentrations. PBDE profile (ΣPBDEs 8-60 ng/g lipid weight) was dominated by the PBDE-209 although other congeners were also detectable. This demonstrates the major ability of these chemicals to accumulate in the Antarctic marine environment. The final outcomes of this study will provide new broad insights on the levels and characteristics of POPs in benthic invertebrates of WAP. They will also contribute to the incorporation of these species to the worldwide monitoring system of POPs, especially in the light of the WAP being considered as a region subjected to some of the world's highest climate change impacts.

**7 Quantifying Variability of Laboratory Bioaccumulation Tests**

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Bioaccumulation tests are a critical component of regulatory dredging evaluations. Standard bioaccumulation tests conducted in accordance with procedures outlined in the Ocean Testing Manual (OTM) and the Inland Testing Manual (ITM) utilize *Macoma nasuta* (a clam), *Alitta virens* (a polychaete) for marine tests and the oligochaete *Lumbriculus variegatus* for freshwater tests. Tests are designed to detect statistical differences in tissue residues of dredged and reference sediment exposed test organisms. However simple statistical differences may not equate to biologically relevant differences, therefore it is important to understand the role of variability associated with test design (laboratory exposure and tissue chemistry) and provide guidance to ensure appropriate expenditure of resources for additional analysis only when biologically relevant differences have been established. While many labs have demonstrated capability to conduct bioaccumulation tests and many labs have demonstrated capability to quantify tissue residues, the interlaboratory variability associated with conducting exposures and quantifying resulting tissue residues in 28-day bioaccumulation tests has never been addressed. To quantify variability of laboratory bioaccumulation tests, the USACE-ERDC, the USGS-CERC and two commercial bioassay laboratories (EA and EcoAnalyst) participated in an interlaboratory evaluation. Marine (*M. nasuta* and *A. virens*) and freshwater (*L. variegatus*) species were exposed in standard 28-day bioaccumulation tests to a marine and freshwater sediment , respectively, contaminated with low levels of PCBs(1-2ppm total PCBs) and PAHs (2-5 ppm total PAHs). At test termination all participating labs provided resulting tissues to ERDC's lab for analysis to enable quantification of exposure variability. In a separate, parallel experiment, the ERDC conducted a second set of 28-day *M. nasuta* and *A. virens* bioaccumulation tests utilizing the same marine sediment and exposure conditions and provided splits of the resulting tissue composite to ERDC's in-house lab and three commercial analytical laboratories for purposes of quantifying analytical variability. Results of interlaboratory comparisons (exposure and analytical) will be presented and the implications for determining biologically relevant differences in 28-day bioaccumulation tests discussed.

**8 Placement of Dredged Sediments in Aquatic Ecosystems: Interpreting Contaminant Bioaccumulation**

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The potential bioaccumulation of sediment-associated contaminants from navigation-dredged material remains a primary source of ecological concerns associated with aquatic placement. Bioaccumulation bioassays using infaunal organisms exposed to dredged material and reference sediment are used in evaluations to assess the potential for contaminant-related impacts. To aid in interpreting bioassay results, statistical inferences and numerous assessment factors are recognized by the USEPA/USACE Ocean Testing Manual (OTM) and Inland Testing Manual (ITM) guidelines; however, detailed information for applying these factors to inform ecological risks are lacking. The goal of this study was to provide detailed guidance for evaluating the biological significance of contaminant bioaccumulation. Comparisons of the magnitude of differences (MODs) of tissue residues is one of the stated assessment factors for which there are limited data. Topic areas considered in this study to offer evidence-based information for applying MODs included 1) bioassay coefficient of variance [CV]), 2) bioaccumulation case studies from three geographic regions within the USA, and 3) reported uncertainty factors relevant to bioaccumulation endpoints. Based on peer-reviewed data and dredged material evaluations, within-sample variability of tissue concentrations were relatively low among testing organisms for a number of commonly assessed constituents (e.g., PCBs, PAHs, metals, butyl tins). For example, within-sample CVs for total PCB (n=12 congeners) tissue concentrations (n=5 replicates) ranged from 5-49% for 28-d standardized bioassays using worms (*L. variegatus* and *A. virens*) and 5-15% for bivalves (*M. nasuta*). Hypothesis-based statistical comparisons ($p< 0.05$; $\alpha=0.05$) were generally able to detect statistically significant differences among tissue concentrations with relatively low MODs (< 2 fold difference) for a number of bioaccumulative contaminants of concern (e.g., PCBs, PAHs, metals, butyl tins) measured in standardized testing organisms (e.g., *M. nasuta, L. variegatus,* and *A. virens*). These results indicate the importance of defining the associated uncertainty for translating bioaccumulation results from the laboratory to the field. Results from this study will provide information and resources to aid in the application of the OTM and ITM assessment factors (e.g., MODs) to inform realistic predictions of biologically significant bioaccumulation.

## Advances in Sediment Quality Assessment for Regulation and Management

**9 Solid-Phase Microextraction Sampling for Quantifying Polycyclic Aromatic Hydrocarbon Concentrations in Porewater to Monitor Sediment Cap Breakthrough**

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Monitoring the integrity of sediment caps is critical to ensuring their long term functionality – isolating contaminants of concern and preventing contaminant migration through the cap and into the surrounding environment. *In situ* solid-phase microextraction (SPME) sediment porewater samplers can quantify upward or downward flux of contaminants through cap materials, thus serving as an "early warning" monitoring tool for cap breakthrough. At the Pacific Sound Resources (PSR) Superfund site located in Elliot Bay in Seattle, WA, in situ SPME samplers were used to monitor the potential migration of polycyclic aromatic hydrocarbons (PAHs) through the sediment cap placed over contaminated subtidal sediments. Concentrations of 18 PAHs were measured in sediment porewater under the cap, at various depths within the cap, and at the sediment-water interface. In 2011 and 2018, SPME samplers were deployed to a depth of 34 inches below the sediment surface for 14 days at 25 cap locations including several potential groundwater pathways. In 2011, PAHs were detected in nearly all depth intervals; however, no porewater results for any PAHs exceeded the Ambient Water Quality Standard (evaluation criteria used in the Second Five Year Review). PAH concentrations in the lower portions of the cap porewater were often very low (near the limits of detection), suggesting no upward PAH migration. However, for some PAHs (e.g. chrysene, fluoranthene, and pyrene), higher porewater concentrations were observed near the sediment cap surface, suggesting potential top-down migration from surface water or recent deposition. Data from the 2018 and 2011 SPME deployments will be compared to identify cap changes over time. The PSR case study illustrates how polymeric porewater samplers can directly support remedy decision making by assessing through-cap contaminant migration and cap functionality over time.

**10 Effect of Aging on Bioavailability of Legacy Contaminants in Sediment**

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Hydrophobic legacy contaminants like dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls (PCBs) were banned almost half a century ago. While their residues still remain in many environmental compartments, they have undergone extensive aging and likely have much lower bioavailability as compared to fresh residues. However, risk assessment relies heavily on the use of total chemical concentration, rather than accounting for the age-diminished bioavailability, likely leading to overestimated risks. In this study, we used 24 h Tenax desorption to measure the potential bioaccessibility of DDTs and PCBs in two sediment cores taken from the Palos Verdes Shelf Superfund site in the Pacific Ocean. Bioaccessibility estimated by 24-h Tenax-aided desorption ($F_{24h}$) decreased in the order of DDD > DDE > DDT for DDT derivatives, and PCB 52 > PCB 70 > PCB 153 for PCB congeners, showing a negative correlation with their log $K_{ow}$. Due to the extensive aging, $F_{24h}$ values were < 20% of the total chemical concentration for most contaminants and < 5% for DDT, DDE and PCB 153, suggesting that aging greatly diminished their bioavailability. However, a quantitative relationship between $F_{24h}$ and sediment age along the vertical profile was not found, likely because the contaminant residues had undergone aging before their offsite transport and deposition onto the ocean floor. Bioaccumulation by marine polychaete *Nereis virens* was measured after the worms were exposed to the aged residues and the derived BSAF was compared to that from fresh-spiked sediment. BSAF was markedly smaller for the aged residues than for the fresh spiked compounds. As the use of man-made chemicals such as DDT and PCBs was discontinued many decades ago, the reduction in their bioavailability due to aging may be universal and should be taken into consideration to avoid overly conservative risk predictions or unnecessary mitigation interventions.

**11 Assessing trace element bioavailability in oxic and anoxic sediments through the use of a biomonitor**

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Assessing the bioavailability of trace elements in sediments is important in ecological risk assessments designed to protect benthic communities and the predators that feed on them. The distribution, form and bioavailability of trace elements in sediments are largely influenced by the presence of oxygen. Chemical methods and models have been developed to assess trace element bioaccumulation and toxicity in oxic and anoxic sediments; however, such predictions tend to be approximate at best. A direct mean of estimating contaminant exposure is to measure trace elements in benthic animals themselves. Good candidates for estimating and comparing trace element bioavailability in oxic and anoxic sediments are larvae of the insect *Chironomus* (Chironomidae, Diptera) because larvae of some species are known to feed on oxic sediments while others on anoxic sediments. The possibility of using *Chironomus* larvae as sediment biomonitors was evaluated by determining if larvae take up the majority of their trace elements from oxic and anoxic sediments rather than from the overlying water that they pump through their tunnels in sediments for respiratory purposes. For this, we compared concentrations of trace elements in *Chironomus* larvae collected in lakes from Rouyn-Noranda (QC) and Sudbury (ON) to those in water (total and free ion) and in oxic and anoxic sediments from where they were collected. Overall, our analyses indicate that *Chironomus* larvae take up the majority of their trace elements from sediments; and thus, can be used to assess the bioavailability of trace elements in sediments. Given that *Chironomus* larvae can be used as sediment biomonitors, we evaluated if the bioavailability of trace elements differs between oxic and anoxic sediments in Rouyn-Noranda and Sudbury lakes. Comparisons of trace elements in larvae feeding on oxic sediments to those in larvae feeding on anoxic sediments collected from the same location indicate that the bioavailable concentration of arsenic, barium, cobalt, copper, manganese and nickel do not differ between oxic and anoxic sediments. However, they indicate that selenium is more bioavailable in anoxic sediments than in oxic sediments; whereas, cadmium and in some lakes zinc, are more bioavailable in oxic sediments than in anoxic sediments.

**12 Assessing avoidance and other behaviors in development of a sediment avoidance test for assessing remediation of contaminated sediments**

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Since its development in 1996, versions of an earthworm avoidance test have seen wide and successful use in assessing contaminated soils, from both toxicological and ecological endpoints. Avoidance of contaminated sediments by aquatic macroinvertebrates has a similar potential to assess degree of contamination and success of methods for remediation of contaminated sediments (such as sediment amendments) at a range of sites (Superfund, Great Lakes AOCs, Mining sites). We assessed the use of the freshwater aquatic macroinvertebrates (*Chironomus dilutus* larvae, *Hyallela azteca*, and *Lumbriculus variegatus*) used in standard EPA methods for sediment toxicity and bioaccumulation (EPA Methods 100.1, 100.2, and 100.3) for use in sediment avoidance testing. Early testing has focused on metals-contaminated sediment. We found evidence of increased sensitivity vs. standard toxicity tests. Both *Hyallela* and *Lumbriculus* avoided metals-contaminated sediment at levels of contamination that caused weight loss but did not show statistically significant lethality in standard 7-day sediment toxicity tests with *Chironomus*. Experimental results, as well as available literature, show that behaviors other than avoidance of contaminants (feeding habits, predator avoidance, mating, phototactic responses to light) must be taken into account in choosing suitable test organisms, developing test procedures, and interpreting results. For instance, *Hyalella* and *Lumbriculus* both showed some evidence of negative phototaxis in absence of substrate. However, with introduction of other physical factors and associated behaviors, the picture changes. With sediment present in the test chambers *Hyalella* seemed to show a positive phototaxis while *Lumbriculus* showed no preference for light or dark sides of the chambers. Our observations indicate that lack of sufficient movement as well as tube-building behavior of *Chironomus* larvae make this organism less than ideal for avoidance tests. Choice of reference sediment is discussed. Though these tests are relatively short (24-48 hours), with a large enough difference in organic content between test and reference sediments, feeding behavior may come into play and cause preference for the sediment with higher organic content and make interpretation of results more difficult.

**13 Refinement of in-situ Toxicity Identification Evaluation (iTIE) System for Assessing Contaminated Sediments, Remediation, and Source Identification**

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It is difficult to assess the toxicity of a single stressor and establish a strong stressor–causality link when multiple stressors coexist. The USEPA's Toxicity identification evaluation (TIE) methodology uses a series of chemical and physical manipulations to fractionate compounds within a matrix and systematically identify potential toxicants. The TIE provides useful information; but, lacks ecological realism as it is subject to laboratory-related artifacts. The in situ TIE (iTIE) technology is a biological-chemical fractionation system that systematically identifies causes of toxicity. The iTIE has undergone a number of iterations to increase the robustness and reliability of the novel technology. The 2018 prototype consists of a rectangular unit capable of housing an array of iTIE units. Each unit is equipped with an organism exposure chamber, a smaller chamber filled with a resin absorbent to fractionate porewater, surface water or effluent passing through the organism chamber, and a connection to a water collection container. A secondary containment unit is used to house the electronic controller along with other components which connects to the primary iTIE housing array. The iTIE system was deployed to a depth of 3 meters and evaluated in streams and marine harbors. The system assisted weight-of-evidence studies to assess stream impairments, wastewater effluents, porewaters and groundwater-surface water interactions using multiple species. Chemical analyses of water and iTIE chemical sorptive resins confirmed lethal to sublethal responses. This latest iTIE prototype provides a robust technology that improves stressor-causality linkages.

**14 Identifying key toxicants in sediment using an integrated method of TIE and EDA: A case study in Guangzhou, China**

*J. You, Jinan University / School of Environment; H. Li, Jinan University; F. Cheng, Jinan University / School of Environment*

Prioritizing toxicants is of a great challenge in ecological risk assessment for sediments in urban waterways which are seriously polluted by multiple stressors. Identifying key toxicants is an indispensable step in prioritizing toxicants concerning environmental risk assessment, management and policy decision. Two approaches, namely toxicity identification evaluation (TIE) and effect-directed analysis (EDA) have been developed to diagnose the causes of sediment toxicity. Conventional sediment TIEs take the advantage of environmental relevance by using whole organism bioassays yet suffer from lack of effective methods for pinpointing main contributors from a universe of organic chemicals. Alternatively, EDA is a powerful tool in identifying causes of sediment toxicity with sophisticated fractionation and chemical analysis methods, but it is usually short of environmental relevance due to the use of in-vitro bioassays and exhaustive solvent extraction. To better understand the cause of sediment toxicity in urban waterways in Guangzhou, China, an integrated method of TIE and EDA was developed. Whole-sediment TIE in combination with bioaccessibility-based extraction found that sediment mortality to the benthic invertebrate, *Chironomus dilutus* was caused by organics and metals jointly and organic pollutants contributed to the mortality for all samples. To better elucidate the roles of non-target organic contaminants in sediment toxicity in these sediments, EDA tests were performed with midge mortality and cell viability as the endpoints. To better understand the mode of action of the toxicants, four cell lines (HepG2, MCF-7, A549 and SH-SY5Y) were used to distinguish toxicants related to metabolism dysfunction, endocrine disruption, respiratory toxicity and neurotoxicity, respectively. All test sediment samples showed significant cell proliferation of SH-SY5Y cell line, but no effect on HepG2 and A549 cell lines. The toxicants were identified in the toxic fraction with GC-MS-MS after separating the extracts to 35 fractions using NPLC. The results were confirmed by midge toxicity testing. The proliferation of SH-SY5Y proliferation was partially explained by oxidative stress. In conclusion, an integrated method of TIE and EDA would provide an environmentally relevant and toxicant specific approach to effectively determine causality of sediment toxicity by combining the advantages of the two methods.

**15 Comparator Site Selection to Inform Screening-Level Causal Assessments**

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Sites in poor ecological condition often require causal assessment to determine appropriate follow-up steps. A key component of this process is to identify a series of ecologically similar sites that are used to assess potential stressors at the impaired site. comparator sites should: 1. be capable of supporting similar biota to the impaired site; 2. consist of sites from a similar environmental setting; 3. comprise a gradient of biotic condition; and 4. contain enough sites to assess variability. There are number of methods for selecting groups of comparator sites to be used in a causal assessment. In the present study, we compared the outputs of two similar, but distinct approaches that were applied across a large number of degraded stream sites in Southern California. One approach selects comparator sites based upon their natural environmental setting (e.g., slope, geology, elevation) and the other uses estimates of expected biological similarity (Bray-Curtis dissimilarity of modelled biotic assemblages). Both approaches were used to generate groups of comparator sites for 81 different stream-segments that have degraded benthic macroinvertebrate assemblages. The groups of comparator sites generated by each approach were evaluated against our comparator site suitability criteria to determine the performance of each approach. The groups of comparator sites identified by both approaches fared well against the four suitability criteria listed above, with greater than 80% success (i.e., comparator sites that passed any single criterion) by both methods in each criterion. The results of this study suggest that both methods – environmental similarity and expected biological dissimilarity – produced good groups of comparator sites in a quantitative, rapid, and conceptually transparent fashion at the scale needed in a large, complex monitoring and assessment program, with each method having subtle advantages for specific applications. The success of these methods in turn sets the groundwork for developing a novel screening-level approach to causal assessment that can be used to quickly diagnose stressors and prioritize problematic waterbodies for follow up from the appropriate regulatory and regulated agencies.

### 16 Stream Pollution Trends Program (SPoT): California Sediment Contaminant and Toxicity Monitoring Related to Land Use

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The Stream Pollution Trends Program (SPoT) monitors the health of 100 California watersheds through measurements of sediment toxicity and contaminants. The focus of the program is trend monitoring based on land use, particularly developed, agricultural and open lands. Developed land area in California increased by 3.7% between 2001 and 2011, and the net increase in impervious surface was 8.8%. Among the developed land uses, developed open space decreased, but there were substantial increases in medium and high impact development. Increases in developed land have coincided with decreases in scrub, grasslands and agricultural lands. Since its inception in 2008, SPoT has identified significant increasing trends in current-use pesticides, heavy metals and the PBDE flame retardants in urban areas. Some individual pyrethroid pesticide concentrations are significantly increasing in all three land use areas, whereas individual metals such as copper are only increasing in open areas. Concentrations of legacy contaminants, such as DDT and PCBs are low at SPoT sites, but significantly decreasing trends are not discernable. Trends in toxicity, measured as survival of laboratory test organisms (the amphipod *Hyalella azteca*) in ambient sediment samples, have been decreasing in open areas, but are stable statewide with significant toxicity observed at approximately one in five sites. The highest incidence and magnitude of toxicity has been associated with urban land use and pyrethroid pesticides. SPoT recently expanded its analyte list to include the urban-use pesticide fipronil, and has added another test organism (the midge *Chironomus dilutus*) to assess potential impacts of this pesticide. Although concentrations of fipronil remain below toxicity thresholds, three years of paired monitoring with the two organisms at urban sites show a decrease in amphipod toxicity and an increase in midge toxicity. SPoT will continue to adapt to identify trends in emerging contaminants through the introduction of new analytes, toxicity test organisms and novel monitoring methods, such as bioanalytical tools and non-target analysis.

## Chemical, Biological and Instrumental Methods for Detecting Harmful Algae Blooms and Their Natural Toxins

### 17 Monitoring Approaches and Methods for Multiple HAB Toxins Across the Freshwater-Marine Continuum in Coastal California

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The conventional focus of HAB monitoring programs has been to monitor and analyze toxins according to the waterbody type sampled, either as marine toxins or freshwater toxins, but not both. However, recent studies have shown that cyanotoxins have far reaching-effects downstream of their freshwater origin in brackish and marine waterbodies. These studies underscore the importance of inland waters as potential conduits for transfer of freshwater toxins to the marine environment, and highlight the importance of a comprehensive, multi-toxin (both marine toxins and cyanotoxins) approach to monitoring throughout the freshwater-marine continuum. There is a new recognition that management and mitigation of HABs needs to occur cohesively across waterbodies within freshwater, estuarine and marine environments due to hydrologic interconnections and toxin impacts downstream of bloom origins. As such, HAB monitoring approaches and methodologies have been developed specifically to address the challenges of comprehensively measuring all types of HABs across a variety of waterbodies. This presentation will focus on the combination of water sampling methodologies, both for toxins and HAB organisms, that have been developed to address this need and have been employed throughout coastal California in regional monitoring and specialized research studies. Passive sampling devices, specifically Solid Phase Adsorption Toxin Tracking (SPATT), have proven to be a critical method for HAB monitoring at the land-sea interface since SPATT provides time-integrated and multi-toxin measurements across all waterbody types. The combination of passive sampling with traditional grab sampling will be discussed, along with the importance of dissolved toxin measurements, often overlooked and under sampled in current ambient monitoring programs in CA. HAB organism identification methods can be accomplished through a combination of methods depending on the specific monitoring needs. The relative abundance index methodology uses microscopy and provides a rapid assessment of HAB genera/species, which can be used to inform event response and to determine the toxins analysis needs. Molecular methods, such as DNA sequencing, can provide more in-depth and quantitative HAB identification. Preliminary comparative studies of both relative abundance microscopy and DNA barcoding indicated a good agreement of broad community composition results.

### 18 Estimating Cyanotoxin Occurrence with Real-Time Water-Quality Data: Case Studies in Kansas and Ohio Lakes and Rivers

*J. Graham, G.M. Foster, D. Francy, T.J. Williams, US Geological Survey*

Toxins produced by cyanobacteria (cyanotoxins) are increasingly a global concern in waterbodies used for drinking water and recreation. Early notification of cyanotoxin occurrences would allow proactive, rather than reactive, management approaches to ensure public health protection. Water-quality data collected continuously and transmitted in near real-time provide a useful indicator of changing water-quality conditions that may affect drinking-water treatment processes and recreational use of waterbodies. Models developed using continuous water-quality data have shown promise as tools to estimate the probability of cyanotoxin occurrence and concentration. Model outputs can be delivered through a variety of platforms to provide timely and relevant public health information to water resource managers. Four case studies, representing seven study sites, conducted by the U.S. Geological Survey in cooperation with local and state partners, from lakes and rivers in Kansas and Ohio will be used to demonstrate the development, refinement, and implementation of water-quality indicators and models to estimate occurrence and concentration of the cyanotoxin microcystin. Logistic regression models to estimate probability of occurrence and/or linear regression models to estimate concentration were successfully developed at all study sites. Models and explanatory variables were site specific. Chlorophyll and phycocyanin fluorescence were included in models most often and streamflow, turbidity, pH, and dissolved oxygen also were useful indicators at some sites. In one case study (Cheney Reservoir, Kansas), models were initially developed in 2013 and have performed well over time. In contrast, in another case study (Kansas River, Kansas), models initially developed in 2016 did not perform well in subsequent years, likely because of flow-driven environmental conditions in this system. In three of the four case studies, water-quality tools have been used with other information to inform management decisions. A real-time model is being validated for a Lake Erie drinking-water intake site in 2018. These case studies demonstrate the strengths and limitations of near-real-time notification tools that indicate when water-quality conditions are changing rapidly or are likely to cause cyanobacteria-related events.

**19 Spatiotemporal variability of cyanobacteria and cyanotoxins, and the potential for sensor-derived recreational advisory status**

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Cyanobacteria and associated toxins in Milford Lake, Kansas, have been determined to be the cause of both human illnesses and animal deaths, and have had an economic impact on surrounding areas. The lake has been under recreational warnings because of high concentrations of the cyanotoxin microcystin every summer since 2011. Extreme spatiotemporal variability in cyanobacteria and microcystin in Milford Lake and other recreational waterbodies poses unique challenges to collecting representative samples for scientific study and public health protection. During the summers of 2015 through 2018, the U.S. Geological Survey, in collaboration with the Kansas Department of Health and Environment (KDHE), collected continuous data from a combination of time-lapse cameras and fluorescence sensors in Milford Lake. These data, along with water samples collected for laboratory analysis, were used to quantify the spatiotemporal variability of cyanobacteria and microcystin. The KDHE monitors cyanobacteria and microcystin in Kansas lakes and is responsible for issuing public health advisories and warnings when concentrations exceeded state-established guidance values. The main objective of this effort was to inform monitoring strategies for public health protection. Time-lapse camera data showed that near-shore accumulations of cyanobacteria can appear and disappear within minutes. Although cyanobacterial biomass and microcystin concentrations often varied by orders of magnitude between near-shore and off-shore locations, the shore-based sampling strategy employed by KDHE to issue public health advisories was consistently representative of the entire lake. During the summers of 2016 through 2018, surrogate microcystin models were developed and used to quantify spatiotemporal variability and evaluate near-real time estimates of microcystin concentrations for utility as a management tool. Exceedance of KDHE guidance values often changed from no advisory to watch and/or warning over the course of a day because of the variability in cyanobacterial biomass and microcystin concentrations caused by bloom development and dissipation. Continuous water-quality monitors may be useful in informing public health decisions in lakes with variable cyanobacterial bloom conditions. However, site-specific models need to be developed, and best practices for using continuous water-quality monitors to inform cyanobacterial bloom management strategies need to be established.

**20 Determination of multiple classes of cyanotoxins in water and fish tissue using liquid chromatography tandem mass spectrometry**

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Current analytical methods for cyanotoxins typically detect discrete classes of cyanotoxins in one matrix and rarely include saxitoxin, a select agent. Thus, a multi-toxin screening method for microcystins (MCs), nodularin (NOD), anatoxin-a (ANA), cylindrospermopsin (CLD), and saxitoxin (SAX) was developed to monitor target analytes in environmental water and fish tissue. Due to the broad range of cyanotoxin physiochemical properties, two separation techniques, hydrophilic interaction liquid chromatography (HILIC), corresponding toANA, CLD, and SAXand reverse phase liquid chromatography (RPLC), corresponding to MCs and NOD were paired to improve isolation and detection. Likewise, isolation of target analytes from water was achieved using the Waters Oasis HLB (RPLC method) and the Supelclean ENVI-carb (HILIC method). The large variation in $pK_a$and lipophilicity of cyanotoxins led to a systematic study of extraction behaviors using different solvent systems comprised of varying ratios of acetonitrile, nanopure water, and aqueous 0.1%formicacid to optimize extraction from fish tissue corresponding to the HILIC or RPLC methods. Further, commercially available isotopically labeled internal standards (IS) for microcystin-LA, LR, RR, and YR were used for the first time to correct for matrix effect and extraction bias of MCs. This protocol further evaluates the use of a zwitterionic HILIC analytical column to separate ANA, CLD, and SAX simultaneously, for the first time. Recoveries in water ranged from 53 – 98% and from 45 – 103% in fish tissue. Method LODs ranged from 4.0 to 80 pg (0.004-0.08 ng $mL^{-1}$), LOQs ranged from 10 to 280 pg (0.01-0.28 ng $mL^{-1}$), and MDLs ranged from 80 to 960 pg (0.08-0.96 ng $L^{-1}$) in water and 120 to 700 pg (0.12-0.70 mg $kg^{-1}$) in fish tissue.An ion suppression ranging from -22% to -77% was observed for MCs and NOD in water, with IS corrections showing improvement where ion suppression and enhancement ranged from -4.2% to +5.4%. Similar to water, ion suppression for fish tissue ranged from -26% to -58%, which was improved with IS correction, where relative values ranged from -17% to +17%. This method was subsequently used to screen for target analytes in water and fish from a caged fish study staged in a hypereutrophic impoundment during summer and winter. MC-LA and LR were detected in all water samples from both sampling periods, however no cyanotoxins were observed in fish tissue.

**21 High Frequency Monitoring of cyanoHABs Dynamics**

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USEPA's Office of Research and Development has partnered with the Clermont County, OH Water Resources Department in an on-going study to assess HAB trends and develop monitoring tools and approaches. Lake Harsha, a multi-use reservoir and primary drinking water source in southwest OH, has experienced an increase in cyanoHAB frequency and intensity over the past several decades. The goal of this work is to develop relationships between HAB indicator measures and cyanotoxin occurrence which can provide time-relevant information regarding source water quality for DWTP operators and other public health stakeholders. In order to characterize the cyanobacterial population and both intracellular and extracellular cyanotoxin production, an intensive sampling regime was implemented beginning in 2015. High frequency (HF) monitoring (in-vivo fluorescence and physico-chemical parameters) was used to determine the timing and rate of discrete sampling of cyanotoxins (LC-MSMS analysis of select MC congeners and a total MC surrogate, cylindrospermopsin, and anatoxin-a, MC ELISA), molecular indicators, taxonomic enumeration, and nutrients, varying from biweekly, to daily collection during the observed bloom peak. Resulting data provide a time-series of the cyanobacterial population dynamic and greatest periods of cyanotoxin production. HF data coupled with cyanotoxin analyses demonstrate the utility of HF data for tracking the cyanoHAB status of the reservoir. It is also apparent that cyanotoxin concentrations may potentially be underestimated if cyanotoxin sampling is not coupled with bloom status. An overview of data and observations from 2015 to the present will be presented.

**22 Use of passive samplers for the detection of extra cellular algal toxins in stream mesocosms, lakes and streams**

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Freshwater Harmful Algal Blooms (HABS) occur frequently throughout the US and many parts of the world. Most of the emphasis on freshwater HABs has been on reservoirs and lakes with recreation and drinking water uses. However, there is mounting evidence that attached benthic cyanobacteria may be increasingly dominating the benthic periphyton community and contributing harmful toxins to streams and rivers. For the past 3 years the USEPA has been testing the consequence of nutrient addition into stream mesocosms. Macroinvertebrate and periphyton communities have been assessed using various measures for their responses to various concentrations of nitrates, phosphates and combination of nitrate/phosphates. At certain doses of these nutrients, cyanobacteria tend to dominate the benthic community. The USEPA and their collaborators have also been studying a HAB-impacted lake (Lake Harsha) for the past 7 years. Most HAB detection methods use discrete water sampling for toxin measurements and cyanobacteria identification. However, newer methods have been developed to detect toxins in California rivers using passive samplers called Solid Phase Adsorption Toxin Tracking (SPATT). A study conducted in China by North Carolina State University researchers has compared several different passive samplers in China, including SPATTs, Polar Organic Chemical Integrative Samplers (POCISs) and other novel Passive Sampler Devices (PSDs). The results indicate that Large Format non-selective PSDs (LF nsPSDs) performed better than other PSDs in recovering microcystins. The purpose of this study is to evaluate SPATTs and LF nsPSDs to determine their performance in determining extracellular algal toxins in both stream mesocosms and within Lake Harsha and its downstream habitats. PSDs were deployed at the end of 16 different stream mesocosms and in two influent mesocosms for a period of two weeks. Every two weeks during the mesocosm study, water samples were collected, PSDs retrieved and then replaced with fresh PSDs. A similar method was followed for PSDs in the lake at 2 locations and at 2 separate depths. Four stream locations were chosen below the outfall of the lake to determine the contribution of lake algal toxins to the downstream stream community. The results of this study will be presented comparing the 2 different types of PSDs and describing the pattern of algal toxins observed in each experimental unit.

**23 Detection of Free and Covalently Bound Microcystins in Sediment and Clam Samples from the San Francisco Estuary**

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Microcystins, a class of hepatotoxins produced by a variety of cyanobacteria species, can significantly impact the health of both animal and human populations. Cyanobacterial blooms are predicted to become more severe worldwide due to rising surface water temperatures and increasing eutrophication of water bodies. Elevated water temperatures and increased UV exposure have also been found to promote growth of toxigenic species. Analytical techniques must allow for accurate quantification of microcystins in biological and environmental specimens to evaluate risk factors, yet this task is challenging as microcystins can become covalently bound to proteins. Thus, methods solely based on solvent extraction may lead to false negative results as they are unable to liberate bound microcystin residues. Here we developed a targeted GC-MS method in which microcystins are oxidized via the Lemieux reaction causing cleavage of the Adda amino acid moiety of microcystins, releasing the compound 3-methyl-2-methoxy-phenylbutric acid (MMPB), and allowing indirect detection and quantification of total microcystin concentrations. The limit of detection and limit of quantification was determined to be 10 ng/g and 50 ng/g, respectively. This method was then used to analyze sediment and clam samples collected from twelve sites in the San Francisco Estuary during May and October of 2017. Our results showed that 40% of the sediment samples were positive for microcystins with concentrations ranging from 16.3 – 151.0 ng/g. All the clam samples had microcystin concentrations below limit of detection. Microcystin positive soil samples were mainly found along the San Joaquin River, suggesting this water body being a potential source for microcystin exposure to the benthic community. This method allows sensitive detection of microcystins and reveals new information for environments at risk.

**24 A nontargeted LC-HRMS approach to correlating differences in HAB chemical 'fingerprints' with toxicity**

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Harmful algal blooms (HABs) are an increasing threat to global water supplies, affecting local economies, public health, and aquatic ecosystems. Their occurrence has historically been studied in estuaries, but HAB incidence in freshwater bodies continues to increase in frequency, duration, and magnitude because of environmental conditions promoted by climate change and urbanization. However, recent work has shown that known toxins fail to completely depict health threats of inland HABs, suggesting that additional compounds responsible for altering bloom toxicity still need to be identified. Traditional bottom-up approaches to toxin identification require time- and labor-intensive studies to systematically identify each component of a HAB mixture and then determine each one's toxicity. We have developed an alternative approach to toxin identification to alleviate the need to identify each component of the complex algal mixture. Instead, the entire chemical 'fingerprint' of algal culture filtrates was examined through nontargeted analysis, and changes in the LC-HRMS heatmap were correlated with changes in toxicity. Changes in the LC-HRMS heatmaps were visualized using relative difference plots which are plots comparing the intensities of a toxic/sample ion map to the intensities of a nontoxic/reference ion map. As a proof of concept, experiments were completed with spiked microcystin samples that contained intentional differences in their chemical 'fingerprint'. Compounds with concentrations changing the most between samples were successfully highlighted while compounds at the same concentration in both samples disappeared from the relative difference plots. *Prymnesium parvum* cultures expressing various levels of acute toxicity were then analyzed using this approach to quickly identify constituents that are produced in higher amounts as a function of increasing toxicity by developing relative difference plots. The highlighted compounds most likely contributing to the difference in acute toxicity expressed by the cultures are considered suspect molecules. Prymnesins were among the highlighted compounds.

## Ecotoxicological Impact of Multiple Stressors in Aquatic Ecosystems

**25 Effects of salmon lice medicine (diflubenzuron) on non-target shrimp populations under climate change: From experiments to population-level endpoints**

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The continued growth of marine aquaculture production has presented the industry with environmental and production concerns, of which the ectoparastic salmon lice (*Lepeophtheirus salmonis*) has gradually become a major problem. A commonly used pesticide against this crustacean is diflubenzuron (DFB), which acts as a chitin synthesis inhibitor and thereby interfere with the moulting stages during sea lice development. However, DFB from medicine feed may also affect non-target crustaceans such as the Northern shrimp (*Pandalus borealis*), which is an economically and ecologically important species in Norwegian fjords. Laboratory experiments have demonstrated that shrimp exposed to DFB through fish feed have reduced survival (ca. 60%) compared to control, in both the larval and the adult stages. Moreover, the effects of DFB exposure are more severe under future climate conditions (higher temperature and ocean acidification). The aim of this study is to make the information on these mechanistic effects more relevant for risk assessment at the population level. We have developed an age-structured population model representing a Northern shrimp population located in a hypothetical Norwegian fjord containing a fish farm, under both ambient and future climates. Our model is based on thorough knowledge of shrimp biology and clear results on toxicological effects from the laboratory experiments. Nevertheless, extrapolating the individual-level effects to the population level poses several challenges. Relevant information on shrimp populations in fjords is sparse (such as abundances, survival and reproductive rates, and density-dependent processes). The degree of exposure to medicine feed at different distances from aquaculture farms is also uncertain. We have therefore developed a set of model scenarios representing different medicine application schemes and different degrees of exposure for the shrimp populations. The purpose of the model is to predict effects of DFB exposure in combination with changed climatic conditions on population-level endpoints such as long-term abundance and age structure, and to assess the risk of population decline below threshold abundances.

**26 Ecotoxicoproteomics provides mechanistic insights into the alleviating effect of ocean acidification on mercury toxicity in marine copepod**

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Due to human activities, marine animals are suffering from multiple stressors, such as ocean acidification (OA) and mercury (Hg) pollution, for many generations; however, the mechanisms-of-action for long-term multigenerational exposure is poorly known, especially concering the interaction. Ecotoxicoproteomics is a relatively new displine that applies global proteomic technologies to toxicological studies, and it is aimed to determine critical proteins/processes affected by environmental stressors, hence figuring out the related mechanisms of action in organisms. In this study, using the copepod *Tigriopus japonicus* as a model species, shotgun-based quantitative proteomics, combined with phenotypic trait observation, is performed to investigate the biochemical mechanism about the effect of OA on Hg toxicity in marine animals under four generation exposure. Physiological observation analysis showed that OA significantly decreased Hg toxicity to reprodution in the copepod, however, which was resilient to reduced pH. Our proteomic analysis demonstrated that OA stress increased energy yielding in the copepod mainly via increased protein assimilation and proteolysis as a compensatory reaction, consequently rendering its physiological robustness to decreased pH. In contrast, Hg exposure decreased many critical processes, including ferric iron binding, antioxidant activity, cellular homeostasis, and glutathione metabolism, and these toxic events could translate into population-level responses, namely restrained reproduction in copepods. More importantly, the alleviation of Hg toxicity in *T. japonicus* by OA could be explained by the enhanced lysosome-autophagy pathway proteomes that are in charge of repairing/removing damaged proteins/enzymes under stress. Taken together, ecotoxicoproteomics is a powerful tool to provide molecular understanding about the response mechanisms of marine animals against multi-stressors.

**27 Copper Effects on Swimming Performance and Predator-Prey Interactions of Sailfin Mollies**

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Copper (Cu) has been used over the last 50 years for its fertilizer properties in Florida. In 2005, over 250 thousand hectares in Florida were treated with more than 500,000 kg of Cu. In 2007, Cu contamination was ranked as one of the top-heavy metals accountable for the decrease in water quality in US water bodies by the USEPA. Due to the extensive use of Cu in Florida and its inability to degrade, Cu has accumulated and remains persistent in Florida's aquatic ecosystems. Exposure to Cu has been found to impair neurotransmitter and chemosensory functions in fish. An excellent method to study Cu sub-lethal effects is fish swimming performance. Saltwater intrusion due to global warming is also of concern in South Florida. The goal of this study was to determine the effects of Cu on [1] swimming performance in Sailfin mollies and [2] predator-prey interactions between largemouth bass and Sailfin mollies across a salinity gradient (freshwater and 8ppt saltwater). Fish (n=32) acclimated to FW or 8ppt saltwater were exposed to 7.20 – 8.20 ug/L Cu for 96 h. At the end of the exposure, fish swimming performance was determined by the critical swimming speed ($U_{crit}$, speed at which a fish cannot longer maintain position in the water column). $U_{crit}$was measured after a10 minute period at a water velocity of 10cm/s, followed by speed increases of 2 cm/s every 5 minutes until reaching 22 cm/s, when speed increases occurred every 15 minutes until the fish stopped swimming. The protocol was replicated to obtain Repetitive $U_{crit}$. Swimming performance ($U_{crit}$& Repetitive $U_{crit}$) was measured again after a 4-week-depuration period to determine if the fish were able to recover. In a separate study, Sailfin mollies (n = 60) were also exposed to 7.20 – 8.20 ug/L Cu; at 96 h, predator-prey interactions were assessed. In Phase 1, Sailfin mollies were presented to visual, chemical, or both visual and chemical cues and tested on predator avoidance. In Phase 2, predator-prey interactions were studied by introducing a live predator to determine prey mortality rate. Our results showed that swimming performance significantly decreased after a 96-h exposure and after a 4-week-depuration period, fish were able to return to their baseline $U_{crit}$. Cu had an effect on visual, chemical, and visual + chemical cues on prey in both Phases 1 and 2. Adverse effects of Cu on fish swimming performance and predator-prey interactions can result in a decline in population survival.

**28 Embryotoxicity and oxidative stress of the ternary mixture of aluminum, iron and mercury on Cyprinus carpio and Danio rerio**

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Aluminum, mercury and iron have been found in high concentrations around the world in different water bodies and have been shown to be very harmful for hydrobionts, causing metabolic dysfunctions and toxic effects at various levels, being the embryonic stage one of the most susceptible to damage. Although contaminants in water bodies usually occur in mixtures, few studies are made taking into account the interaction that could exist between them, this being a factor that can modify the toxic response in exposed organisms. *Cyprinus carpio* and *Danio rerio* are species in which the toxicity produced by metals can be observed effectively. In this study, the embryos of both species were exposed to the tertiary mixture of Al, Hg and Fe at concentrations equivalent to the maximum permissible limit for the protection of aquatic life, from fertilization to hatching. During this period, possible congenital malformations were assessed, as well as oxidative stress (degree of lipoperoxidation, content of oxidized proteins and hydroperoxides and activity of SOD, CAT and GPx) at 12, 24, 48, 72 and 96 h. The results obtained show that the mixture of these metals produces modifications to the embryonic development and alterations in the activity of the antioxidant enzymes and increase in the degree of lipoperoxidation, oxidized proteins and hydroperoxides in both species. In *Cyprinus carpio* the increased oxidative effect was observed mainly at 72 and 96 hours. In studies conducted with metals in isolation, it was found that neither iron nor mercury exert toxicity on embryos by oxidative stress, so there is a possibility that some of these metals are exerting an effect of potentiation on aluminum to increase the toxicity in the mixture, since this last metal turned out to be the main element that produced damages in embryos. As for *Danio rerio*, alterations in the activity of antioxidant enzymes and damage to biomolecules were observed in the different exposure times. It is concluded that the effect of the three metals is altered by the interaction between them and that interaction studies are necessary because in the environment the contaminants are in complex mixtures that can change the toxic effect of isolated xenobiotics.

**29 Effects of coal mining-associated stressors on stream fishes**

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Coal mining promotes stream salinization and species imperilment in the central Appalachians, USA. Conductivity-based extirpation thresholds for stream fishes have been derived using field data from this region. However, mining-induced sedimentation and elevated trace element concentrations can confound salinization effects. We developed a mining intensity gradient for watersheds in the Virginia coalfield and assessed multiple levels of ecological organization to increase our understanding of how multiple stressors affect Appalachian stream fishes. Existing data on fish communities, conductivity, and physical habitat were evaluated for 83 streams. We selected 15 streams using a stratified random design and assessed population- and individual-level responses of two native species, *Rhinichthys obtusus* and *Etheostoma flabellare,* to the mining gradient and associated stressors, including sedimentation and aqueous concentrations of major ions and trace elements. Occurrence of *R. obtusus* was not affected by mining intensity. However, the proportion of age-0 individuals was negatively related to mining intensity and also to [Se] in ovary tissue, which was positively related to mining intensity, despite limited detection of aqueous Se (2 of 15 sites). Glutathione peroxidase activity in *R. obtusus* liver tissue was positively related to the mining gradient and ovary [Se], indicating a physiological effect of Se. Presence of *E. flabellare* was negatively related to conductivity, this species was not collected at sites with the greatest mining intensity. The *E. flabellare* sex ratio, as proportion of females, was negatively related to the mining gradient. The greater impact of mining-related stressors on females may reflect greater energy allocation to reproduction. During the *E. flabellare* spawning season, activities of two energy-dependent enzymes, total ATPase in gill tissue and glutathione reductase in liver tissue, were negatively related to the mining gradient. Overall, our results suggest that mining activities negatively affect stream fishes via a trophic-energetic pathway and demonstrate individual- population-level effects prior to local extirpation. Although major ions are a driver of observed relationships between mining and responses by fishes, our results highlight the importance of considering Se bioaccumulation in fishes residing in coalfield streams, as Se appears to be a more significant co-stressor than indicated by measurable aqueous concentrations.

**30 Development of Dissolved Oxygen Criteria in Suisun Marsh, Part of the San Francisco Bay-Estuary Region**

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Suisun Marsh is the largest tidal wetland in California, with a high diversity of aquatic life in its mix of open water sloughs and managed wetlands. The marsh is especially important as a habitat given its location within an urbanized estuary, and downstream of the Sacramento-San Joaquin River Delta which is the focus of large-scale restoration efforts. However, discharges into sloughs and limited flushing have affected dissolved oxygen (DO) levels in some open waters of the marsh, with adverse effects on aquatic life. Other constituents of concern include mercury, nutrients and organic carbon loading. To address these impairments, the San Francisco Bay Water Board has adopted a Total Maximum Daily Load (TMDL) for Suisun Marsh. A key component is a new set of DO water quality objectives for the open waters of the marsh. Here we describe the process used to develop DO criteria in the marsh, based on several refinements of EPA's Virginian Province approach. From the list of species present in the marsh, we identified surrogate species with toxicological data on DO effects. Surrogate species selection was a key step since published toxicological studies on DO are available for a small number of relevant species. Literature data on DO effects were used to define DO criteria for chronic and acute exposure time frames (day, week, and month). This work also made use of continuous DO data from reference stations, collected over a decade, and data on fish abundance in the marsh from monthly trawls conducted over 17 years to evaluate naturally attainable expectations for DO. The new DO objectives are intended to protect against the adverse effects of low DO on survival, growth, reproduction, and behavior of fish in the marsh and to accommodate both spatial and temporal aspects of low oxygen events. This is the first effort since 1975 to refine DO water quality objectives in the San Francisco Bay region, and may have broader application for other tidal marshes on the California coast.

**31 Bioeffects Assessment in Bristol Bay, Alaska**

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A baseline environmental characterization of the northern reaches of Bristol Bay, Alaska was conducted using the sediment quality triad approach (chemistry, toxicity, and benthic invertebrate community structure), along with measures of contaminant body burdens and the characterization of parasites and disease in starry flounder (Platichthys stellatus) and rainbow smelt (Osmerus mordax). The study area was subdivided into 6 strata based on geophysical and hydrodynamic characteristics. Within each stratum, a stratified random sampling approach was used to select sampling sites for surficial sediment. Concentrations of over 150 organic contaminants and metals were analyzed. Ambient toxicity was assessed using two bioassays (Microtox® and sea urchin fertilization and development). Habitat conditions (depth, salinity, temperature, dissolved oxygen, sediment grain size, and organic carbon content) were also measured. The study results indicated that organic contaminants, including polycyclic aromatic hydrocarbons (PAHs), were low relative to NOAA's sediment quality guidelines (SQG). Tributyltin was detected at trace levels only in Dillingham Harbor. Polychlorinated biphenyls (PCBs) and other chlorinated organic contaminants were detected only in trace amounts in the sediment. Sediment metal concentrations were very low; all values were below NOAA sediment quality guidelines (SQGs), except for arsenic. Benthic communities were relatively sparse at most locations due to harsh physical conditions. Species richness and diversity had no correlation to grain size, TOC, depth, or location when outliers were removed. Significant chemical toxicity was virtually absent except for high porewater ammonia levels at selected locations, associated with fish processing waste streams. Contaminant body burdens and histopathological lesions were very low in the fish tested. Except for an occurrence of an external papilloma condition and the observation of mild to moderate accumulation of macrophage aggregates in the spleen and/or kidney in some flounders, the fish were generally healthy and non-contaminated. In general, the bay appeared to be a robust environment with a biologically diverse benthic assemblage. A follow up study may be needed to assess if the ammonia issue has a significant impact on the overall biological system. We also need to investigate the fate of the fish waste to see whether it is taken up by the benthic or the pelagic food web.

### 32 Comparison of Ecologically and Economically Valued Aquatic Organisms in the Analysis of Fungicide Exposure

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The fungicide dicloran is a model compound for analyzing the impacts of pesticide exposure to non-target aquatic organisms, taking external factors such as sunlight, salinity, and sediment into account for the experimental design. Fathead minnows (*P. promelas*) are a commonly used freshwater vertebrate species in toxicological assays, while red swamp crayfish (*P. clarkii*) are a non-traditional freshwater aquatic invertebrate for toxicological assays. Minnows are a beneficial representation of an ecologically valuable organism in freshwater systems and crayfish are not only ecologically valuable in many systems but in regions such as south Louisiana, they are also considered to be an economically valuable organism. Previous studies have shown that dicloran undergoes photolysis in laboratory analyses, degrading via a quinone-hydroquinone pathway. While the photodegradation half-life of dicloran is not impacted by salinity, it does affect the generation of intermediate products. The presence of sediment slows the overall dissipation of dicloran as sediment partitioning acts as a competitive sink against photolysis in the overlying water. Crayfish are benthic macroinvertebrates and therefore are at risk for exposure to pesticide exposure by runoff in the water column and pesticide partitioning into the sediment. Exposure to dicloran and simulated sunlight for fathead minnows estimated a $LC_{50}$ of 0.23 mg/L, with no mortality observed in dark exposures; crayfish exposed to dicloran and simulated sunlight showed more resilience with an estimated $LC_{50}$ between 0.50 and 1.0 mg/L without the presence of sediment. Dicloran has also been shown to be toxic and phototoxic to non-target aquatic organisms including eastern oysters and inland silversides.

## Advancing -omics into Regulatory Frameworks: Case Studies and Perspectives

### 33 Case Study: Estimation of Points of Departure for Estrogen Disrupting Substances in Fish Using Toxicogenomics

*F. Page-Lariviere, D. Crump, J. O'Brien, Environment and Climate Change Canada / National Wildlife Research Centre*

Traditional toxicity methods for ecological hazard evaluation of substances are expensive, time-consuming and focus on lethality and acute effects. Toxicogenomics may represent a viable alternative for estimating conservative points of departure (PoD) for chemicals with potential chronic toxicity. In this case study, we demonstrate that dose-response modeling of the transcriptomic response following short-term exposures can be used to estimate conservative PoDs for estrogen-disrupting substances (EDS). Publicly available transcriptomic datasets (n=6) from fish models (various tissues) exposed to multiple doses (>3) of three EDS (bisphenol A, ethinylestradiol, and diethylstilbesterol) were analyzed using BMDExpress (v.2.2). Our goal was to establish transcriptomic benchmark doses (BMDs) that could be used as PoDs for ecological hazard evaluation. In addition, differentially expressed genes were grouped into biological processes based on gene ontology terms, and process-specific BMDs were determined. To test for potential bias related to data processing, our analysis compared the effect of different normalization (fastlo, cyclic loess, lumi quantile, and vsn) and filter methods (ANOVA and Williams, with/without FDR and/or fold-change filter) on the transcriptomic BMDs. The resulting BMDs were then compared to the empirically-derived apical NOAEL (no observable adverse effect level) of each substance. We found that both normalization and filtering methods affect the final BMDs (approximately 1500-fold difference), accentuating the importance of consistent data processing for inter-study comparisons. BMDs were also impacted by the dose range tested, highlighting a preference for experimental designs containing multiple doses over a wide range of concentrations. Nevertheless, the comparison of most of the transcriptomic BMDs with apical NOAELs revealed close similarities between methods, suggesting that toxicogenomic dose-response modeling has the potential to be a protective decision-support tool for compounds with chronic toxicity, such as EDS.

### 34 The Environmental-Predictive-Information-Connectivity map (EPIC-map)

*C. Iosif, University of Liverpool; P. Antczak, University of Liverpool / Institute of Integrative Biology*

The adverse outcome pathway framework (AOP) has provided the ecotoxicology community with a great framework to conceptualise the knowledge generated in recent years. Particularly, with the advent of 'OMICs' approaches, knowledge has been generated on a large number of environmental stressors. Central to this framework is the AOPWiki which currently holds just over 200 different AOPs with varying amount of detail. Although AOPs are inherently stressor-free, i.e. an AOP is defined by the progression from a molecular initiating event (MIEs) to a phenotypic outcome through key events (KEs), the identification and development of AOPs is highly guided by the selection of stressors. In addition, to identify what MIEs a given stressors might activate, and whether it is sufficient to cause the observed phenotypic effect, can be challenging. To reduce the amount of time to identify suitable MIEs and KEs, given a novel stressor, a data driven approach is required that combines the power of OMICs, QSAR and Read-across approaches in a single easy to use software solution. The EPIC-map addresses this need by developing a single dataset of dose response relationships across more than 150 compounds of environmental concern in *Danio rerio* embryos including any observable adverse outcomes (AOs) and transcriptional response at concentrations below LC5 both linked to the structural features of the stressors. To access this data, we develop a web-based user interface by which the predictive and network models can be easily interrogated either with your own data or by selecting a compound from the pubchem database. To date the EPIC-map contains dose response curves for over 250 individual compounds of which 160 are currently subjected to transcriptomics screening. The aim of the EPIC-map is to provide the user with an easy to user interface to test and identify, *in-silico*, the likely AOPs (including a list of expected AOs), dose response curves and other important characteristics of chemicals that might be useful in prioritization, further testing or other components of environmental science. In this talk we will present the first unveiling of the EPIC-map, its ideas and predictive models that it uses in its current form and how it will integrate the different datasets and approaches to aid in AOP identification.

**35 Pathway-Based Approach for Chemical Classification Using Transcriptome**

*X. Pu, X. Zhang, Nanjing University / Environmental Science*

Deciphering the biological similarity between chemicals is a formidable challenge in read-across. Omics data has shown advantages in inferring high-resolution biological similarity by global molecular patterns. However, the application of omics data for chemical classification is limited due to the lack of in-depth analysis methods of elucidating biological similarity on pathway-based and dose-dependent levels. Here, we proposed a pathway-based framework for chemical classification, which integrates biological potency and enrichment scores of pathways derived from dose-dependent transcriptomics data. A case study was conducted on 17 (non-)genotoxic chemicals. A recently developed reduced human transcriptomics (RHT) approach was used to generate wide dose-dependent transcriptional expressions of 1200 human genes for each chemical, followed by evaluating the performance for chemical classification by each of three typical pathway analysis methods, including point of departure analysis (PODA), over representation analysis (ORA) and gene set enrichment analysis (GSEA). PODA can characterize transcriptomics-based biological potency which can clearly distinguish high potent genotoxicants and low potent negative controls, but cannot discriminate medium potent genotoxic and non-genotoxic chemicals. For characterization of enriched pathways, ORA can only provide statistical significance of pathways which showed poor performance for chemical classification, while GSEA can provide enrichment scores of pathways partially explainable by MOAs of chemicals, but GSEA method lacks potency information to improve classification performance. Integration of PODA and GSEA improves the accuracy of chemical classification, which stably separated all genotoxic and non-genotoxic chemicals under various weight of indexes by using ToxPi. Overall, biological potency derived from PODA and enrichment scores derived from GSEA were proposed to be combined as integrative indexes, which can promote pathway-based approach for chemical classification.

**36 A landscape-based approach to assess impacts from exposure to complex environmental mixtures in the Shenandoah River**

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Aquatic habitats are often contaminated with complex mixtures of chemicals originating from diverse sources. Different sources of contaminants may be associated with different land uses in surrounding watersheds. Thus, land use and other landscape-scale metrics may be informative for understanding patterns of pollution and exposure effects. Here, we employed integrated chemical and biological analyses to determine how mixture composition and exposure effects varied between sites with different land use. Adult fathead minnows (*Pimephales promelas*) were exposed to water from different locations within the Shenandoah River watershed in mobile, in situ laboratories. Exposure locations included agricultural, urban, waste-water impacted, mixed-use, and forested sites. It is reasoned that the association between landscape variables and chemical occurrence depends on the scale at which landscape variables are measured. Therefore, exposures were conducted at the watershed scale, as well as the site scale, with multiple locations within a single river reach. Multiple biological endpoints were measured, including gonadosomatic index (GSI), number of nuptial tubercles, plasma vitellogenin, and hepatic gene expression. Water samples were taken during the fish exposure and analyzed for more than 460 chemical constituents. Each location had a unique chemical profile that was generally consistent with landuse. Whole-organism and molecular responses also differed between the sites. At a waste-water impacted site, organismal and tissue biomarkers of endocrine disruption were significantly affected, including decreased GSI, altered secondary sex characteristics, and decreased sperm abundance. However, molecular biomarkers of estrogen exposureshowed no difference between sites, suggesting that estrogen receptor activation was likely not a mechanism for these adverse outcomes. Fish exposed at an agriculture-impacted site had increased mortality and decreased GSI relative to initial controls, which was accompanied by differential regulation of immune and metabolism related pathways at the transcript level. Lastly, hierarchical clustering of hepatic transcriptome profiles showed a site-specific pattern, demonstrating that sites with differing landuse exert unique exposure effects at the transcript level. The continued study of the relationships between land use, chemical occurrence, and exposure effects is expected to yield predictive tools for risk assessment and management.

**37 Incorporating transgenerational epigenetic inheritance into ecological risk assessment frameworks: A heavy metal case study**

*J. Shaw, CSIRO / Land and Water*

Detrimental heritable modifications to organisms could be induced by chronic exposure to environmental contaminants. However, to what extent transgenerational inheritance occurs is still disputed. We carried out numerous multi-generation epigenetic-focused plant experiments to evaluate transgenerational inheritance. Our experiments extended to the F3 generation, beyond what could be deemed “direct exposure” to F1 and F2 gametes, and also included subsequent non-exposed generations to evaluate transgenerational recovery times. To examine the apparent irreproducibility of current transgenerational studies we assessed these effects in two distinct model plant species under controlled conditions, using two well-studied contaminants: the heavy metals, silver and copper. We collected both morphological data and reduced representation bisulphate sequencing data to assess the prevalence of transgenerational effects. We attempt to correlate epigenetic end points with observable organism changes before determining a need for transgenerational risk management. Furthermore, we propose a regulatory framework and optimal experimental design that enables transgenerational epigenetic effects to be assessed and incorporated into conventional ecotoxicological testing in a more useful manner.

**38 Selenomethionine induced molecular toxicity in the fathead minnow (*Pimephales promelas*)**

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Selenium is a naturally occurring trace element anthropogenically released to the aquatic environment from activities such as mining and fertilizer application. Within the aquatic environment, primary producers convert aqueous phase selenium oxyanions to organic species, including selenomethionine (SeMet), which can bioaccumulate and cause ecological harm, typically at the top of aquatic food webs. Excess SeMet exposure is known to cause several physiological alterations including oxidative stress and altered stress response. Ingestion of elevated concentrations of SeMet can cause direct toxicity to exposed animals, as well as aggregate in the yolks of oviparous animals and potentially lead to complete recruitment failure through embryotoxicity. However, there is still uncertainty regarding the specific mechanisms by which SeMet causes these pathologies. Therefore, the goal of this study was to conduct an in-depth characterization of the molecular mechanisms underlying SeMet toxicities with the aim to identify molecular toxicity pathways that enable prediction of pathologies before they occur. This study exposed adult fathead minnows (*Pimephales promelas*) to graded concentrations of dietary SeMet (1.18 µg/g dry weight control, and 3.88, 8.75, and 29.58 µg/g dry weight treatments) for 28 days in order to determine its effects on biochemical and molecular processes in this species. Thiobarbituric acid reactive substances, reduced to oxidized glutathione ratios, superoxide dismutase, and catalase assays are being employed on liver tissues to determine the extent of oxidative damage, while liver whole transcriptome and proteome analyses are being conducted to determine molecular toxicity pathways characteristic of SeMet exposure in these fish. These results are then intended to validate and inform the development of critical molecular endpoints of SeMet toxicity in fish that will be used to inform the generation of the EcotoxChip (@ecotoxchip), a qPCR microarray screening tool ultimately intended to expedite the risk assessment of chemicals and mixtures of potential environmental concern.

**39 Assessment of the transcriptome in tree swallow (*Tachycineta bicolor*) nestlings from Great Lakes Areas of Concern**

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Polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzo-p-dioxins and dibenzo-furans (PCDD/PCDFs), and a variety of pesticides and other contaminants of emerging concern (CECs) are significant issues in Areas of Concern (AOC) on the Great Lakes. Pharmaceuticals and personal care products have become major CECs with limited knowledge of the effects of dietary exposure to terrestrial animals. Tree swallow (*Tachycineta bicolor*) nestlings were collected from 27 AOCs and nine nearby non-AOC sites from 2010 to 2015. Contaminant analyses and biomarkers were examined in all nestlings collected. In 2016, nestlings were collected from six locations on the Maumee River to evaluate CECs, including pharmaceuticals and personal care products. Transcriptomic responses were evaluated in nestlings collected from selected sites on the Great Lakes and the Maumee River. RNA-Seq library construction and sequencing were done with Illumina chemistries. *De novo* assembly and downstream differential gene expression (DGE) analyses utilized the Trinity platform and its suggested workflow. In addition, a combined *de novo* Transcriptome assembly (CDTA) was used to improve the completeness of the assembled transcriptome. Assembly completeness is 89.2%, as measured via BUSCO using vertebrate single-copy orthologs. We found that the CDTA approach reduced redundancy by 80% in the assembled transcriptome. Differential gene expression was correlated with geological location for the samples collected over the entire Great Lakes area, including within the higher resolution sampling along the Maumee River. Dietary PAHs could explain the separation in biomarkers of oxidative stress and the gene expression of several Nrf2-regulated antioxidant genes. Correspondence analysis was used to describe the correlation between each gene expression cluster with similar molecular functions and the contaminant profiles from the same nestling.

**40 De novo transcriptome assemblies and transcriptional profiling of 16 Fundulus killifish species in response to environmental challenge**

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Transcriptomic resources and methods are increasingly being used for chemical safety testing in a few standard species. With advances in technology, these tools can now be readily developed for diverse species, and thereby enable chemical safety testing that is tailored, for example, to native species of concern or to enable cross-species extrapolation. However, a lack of robust tools for reproducible assembly and annotation of reference transcriptomes, and methods to analyze expression across species in a phylogenetic context that enables direct species to species comparisons, currently limits the science. Here we present a robust and reproducible informatics pipeline for the *de novo* assembly and annotation of transcriptomes from sixteen killifish species from the genus *Fundulus*. We use this matrix of annotations to directly compare multi-species responses to environmental challenge. We discover patterns of shared or diverged gene expression in the context of phylogenetic relatedness in addition to species-specific differences in sensitivity to the environmental challenge. Though our proof of principle includes challenge to salinity, our workflow provides a framework for tool development and cross-species contrasts to any environmental challenge including toxicants. This provides a template for development and deployment of transcriptomics tools and methods to be used for transcriptional profiling in a broad range of species to enable mechanistic insight into responses to environmental change, including pollution. Risk assessors and environmental managers can thereby access insights derived from mechanistic responses of specific and diverse groups of species.

## Bioavailability-Based Aquatic Toxicity Models for Metals

**41 State of the Science of Metals Bioavailability in Natural Waters**

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As part of the SETAC Workshop on Metals Bioavailability, December, 2017 the history of the development of metal bioavailability concepts was reviewed. This presentation gives an overview of metal bioavailability developments for aquatic organisms, development of models and application to bioavailability-based water quality criteria and standards. Metals are widely studied environmental contaminants due to their ubiquity, potential toxicity to aquatic life, and tendency for their aquatic toxicity to vary widely with the chemistry of the surface water in which they occur. The implications of metal bioavailability for ecological risk assessment are large, as it can produce differences in toxicity of more than 100-fold across a range of water chemistries in surface waters. Beginning as early as the 1930s, considerable research effort has been expended in an attempt to document and understand metal bioavailability, as a function of total and dissolved metal, water hardness, natural organic matter (NOM), pH, and other characteristics of natural waters. The growing understanding of these factors, and improvements in both analytical and computational chemistry, led in turn to a series of modeling approaches intended to describe and predict the relationship between water chemistry and metal toxicity, including the Free Ion Activity Model (FIAM), the Gill Surface Interaction Model (GSIM), the Biotic Ligand Model (BLM), and additional derivatives and regression models that arose from similar knowledge. The arc of these scientific advances can also be traced through the evolution of USEPA Ambient Water Quality Criteria over the last 50 years, from guidance in the "Green Book" published in 1968, to metal-specific criteria produced in the last decade. Through time, these criteria have incorporated increasingly sophisticated means of addressing metal bioavailability, as has regulatory guidance developed by jurisdictions across the globe. These actions have shifted the debate toward identifying harmonized approaches for determining when knowledge is adequate to establish bioavailability-based approaches and how best to implement them into regulatory practice.

**42 Metal bioavailability models: Current status, lessons learned, considerations for regulatory use, and the path forward**

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Since the early 2000s, biotic ligand models (BLMs) and related constructs have been a dominant paradigm for risk assessment of aqueous metals in the environment. We review the current status of metals bioavailability models and recommend considerations for their use and refinement. Striking a balance between comprehensive, mechanistically sound models or simplified approaches is a challenge; pH effects as a factor modifying metals toxicity are unique, with multiple possible mechanisms. As such, we doubt it is ever appropriate to lump algae and animal bioavailability models; however it is often reasonable to lump bioavailability models for animals, although aquatic insects may be an exception. Other recommendations include that data generated for model development should consider equilibrium conditions in exposure designs, including food items in combined waterborne-dietary matched chronic exposures. Some potentially important toxicity modifying factors are currently not represented in bioavailability models, and have received insufficient attention in toxicity testing. Temperature is probably of foremost importance; phosphate is likely important in plant and algae models. If empirical bioavailability tools such as multiple-linear regression models and look-up tables are employed in criteria, they should always be informed qualitatively and quantitatively by mechanistic models. If bioavailability models are to be used in environmental regulation, ongoing support and availability for use of the models in the public domain are essential.

**43 Guidance on the Development of Empirical Bioavailability Models for Metals**

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Recently, there has been renewed interest in the development and use of empirical models to predict metal bioavailability and derive water quality criteria. However, there is considerable variability in the conceptual and statistical approaches with which these models have been developed. We review several case studies of empirical bioavailability model development, evaluating and making recommendations on key issues, including: species selection, identification of toxicity modifying factors (TMFs) and the appropriate environmental range of these factors, use of existing toxicity data sets and experimental design for developing new data sets, statistical considerations in deriving species-specific and pooled bioavailability models, and normalization of species sensitivity distributions using these models. We recommend that TMFs be identified from available chemical speciation and toxicity data, and statistical evaluations of their relationships to toxicity. Experimental designs for new toxicity data must be sufficiently robust to detect non-linear responses to TMFs and should encompass a large fraction (e.g., 90%) of the TMF range while avoiding TMF combinations that are unlikely to occur in natural waters. Model development should involve a rigorous use of both visual plotting and statistical techniques to evaluate data fit. When data allow, we recommend using a simple linear model structure and developing pooled models rather than retaining multiple taxa-specific models. We conclude that empirical bioavailability models often have similar predictive capabilities compared to mechanistic models and can provide a relatively simple, transparent tool for predicting the effects of TMFs on metal bioavailability to achieve desired environmental management goals.

**44 Validation of Bioavailability-based Toxicity Models for Metals**

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Environmental agencies world-wide are increasingly incorporating bioavailability-based toxicity models into development of protective values for aquatic life such as water quality criteria, standards, and/or guidelines for metals. Use of such models to develop protective values should be contingent upon their ability to meet performance criteria as specified through a model validation process. Model validation generally involves an assessment of a model's appropriateness, relevance, and accuracy (i.e., Is it "fit for purpose"?). We review existing guidance for validation of bioavailability-based toxicity models, pose questions to be asked in model validation studies, discuss model study type and design considerations, present several new ways to evaluate model performance in validation studies, and suggest a framework for use of model validation in development of protective values for aquatic life. We conclude that model validation should be rigorous, but flexible enough to meet the performance criteria established for the model and fit the model's purpose. Although a model can never be fully validated to a level of zero uncertainty, it can be sufficiently validated to fit a specific purpose. Therefore, the level of support for a model should be presented/reported in such a way that the user can choose the level of acceptability consistent with the model's purpose. Models should be validated using experimental designs and endpoints consistent with the datasets that were used to parameterize and calibrate the model, and they should be validated across a broad range of geographically- and ecologically-relevant water types. Model validation is a continuous and iterative process that will necessarily be constrained by the amount of data available. It is important to recognize that models have a lifecycle and that scientific knowledge will continue to advance along with the collection of additional data. As models advance, they might need to undergo additional validation. In the future, as more models are developed and refined to predict population-, community-, and ecosystem-level effects of metals, validation methodology will have to evolve to meet those needs.

**45 Derivation and Application of Thresholds for Metals Using Bioavailability-Based Approaches**

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An overview will be provided regarding best practices for evaluating bioavailability models for metals for use in the protection of aquatic life. This presentation will include descriptions of the state of the science regarding a) the evaluation and selection of ecotoxicity data, b) the selection of bioavailability models for use in normalization, and c) subsequent application of bioavailability models. While many examples of normalization steps exist worldwide, a scheme is proposed to evaluate and select a model that takes account its representativeness (water chemistry and taxonomic coverage of the ecotoxicity dataset) and validation performance. Important considerations for a suitable model are the quantity of inputs needed, accuracy, and ease of use; all of which are needed to set protective water quality values for metals and to use these values to evaluate potential risks to receiving waters. While the end results of different model application approaches may be broadly similar, the differences in these application frameworks ultimately come down to a series of tradeoffs between who needs to collect the data and use the bioavailability model, the different requirements of spatial scales involved (e.g., regional vs. site-specific values), and a balance between accuracy and expected protectiveness.

Ultimately, understanding the limits and consequences of these tradeoffs allows for balance between accuracy of aquatic life protection and intended levels of protection.

**46 Application of the Biotic Ligand Model (BLM): A Case Study in Assessing Metals Risk to Vernal Pool Species**

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An ecological risk assessment (ERA) is being performed at a former munitions manufacturing site in Connecticut. The majority of the site is forested upland habitat, with limited or no contaminant migration pathways associated with historical activities; aquatic and ephemeral and permanent wetland habitats are also present, including vernal pools. As such, exposure of vernal pool biota to site-related metals is being evaluated as part of the ERA. A screening evaluation of existing wetland and vernal pool surface-water data indicated potential for ecological risks associated with copper, lead, and nickel based on exceedance of applicable ecological screening values, including hardness-based Water Quality Criteria for Aquatic Life (WQCs). However, the initial screening did not account for two site-specific factors in assessing potential risk due to metals in vernal pools and aquatic habitats: site-specific metals bioavailability and site-specific receptors of concern (ROCs), including the obligate vernal pool invertebrate and amphibian species. To account for site-specific metals bioavailability, sample-specific WQCs were calculated based on the application of the Biotic Ligand Model (BLM) for copper. The BLM incorporates site-specific bioavailability-modifying factors such as dissolved organic carbon (DOC), pH and alkalinity. For the existing datasets with estimated DOC and alkalinity, exceedances of BLM-based WQCs for copper were minor, indicating generally limited aquatic bioavailability of copper at the site. To further account for the site-specific ROCs, amphibian-specific No Observed Effect Concentrations (NOECs) were derived based on literature data. Metals exceedances were also generally minor relative to the amphibian-specific NOECs, indicating that the potential risk to an important group of site-specific ROCs is likely limited. Surface-water samples were collected recently in May 2018 to represent current site conditions, to fill data gaps for refined evaluation of bioavailability, and to capture the most relevant season for aquatic exposure of vernal pool species. Ongoing evaluation of the current data includes an initial screening and subsequent refinements using BLM-based WQC for copper and amphibian-specific NOECs. The focus of this presentation will be the results of the BLM-based evaluation of site-specific metals bioavailability and toxicity and consideration of site-specific ROCs for the on-site vernal pools and wetland habitats.

**47 Influence of water chemistry on the chronic toxicity of nickel and zinc to the mayfly Neocloeon triangulifer in natural waters**

*D.J. Soucek, Illinois Natural History Survey; A. Dickinson, Illinois Natural History Survey / Ecology; C.E. Schlekat, NiPERA; E. Van Genderen, International Zinc Association*

Many states currently rely on dated hardness-based criteria for regulation of nickel and zinc in surface waters. Resistance to adoption of criteria based on the biotic ligand model (BLM) is based in part on uncertainty about whether these models can reliably predict toxic effects of nickel (Ni) and zinc (Zn) on diverse assemblages of aquatic biota across the wide range of water quality conditions occurring in the region. Examination of water quality data across different ecoregions within Region 5 (Great Lakes/Upper Midwest) identified two areas with extreme values of several water chemistry parameters: the 'Northern Lakes and Forests' (NF) ecoregion of Minnesota, Michigan, and Wisconsin has a high frequency of low pH/low hardness/high dissolved organic carbon (DOC) waters, and the Corn Belt (CB) ecoregion of Iowa and Illinois has many waters with high hardness, high pH, and high alkalinity together with low to moderate DOC concentrations. Another area of uncertainty in the application of Ni and Zn BLMs is whether models developed based on responses of a limited number of invertebrates and fish can adequately predict toxicity to the full range of aquatic invertebrate taxa. The objective of this research was to estimate the representative Ni and Zn sensitivities for the mayfly, *Neocloeon triangulifer*, based on chronic tests with four natural waters, two from NF and two from CB. We also sought to evaluate whether BLM models can explain variation among Ni and Zn effect concentrations across a range of water chemistries as effectively for mayflies as for *Ceriodaphnia dubia*. The mayfly was more sensitive to Zn than to Ni, with EC20s for Ni being 2 to 6 fold higher than those for Zn. Dissolved organic carbon appeared to have a strong influence on toxicity to the mayfly. For example the Ni EC20 in the high DOC low hardness NF waters were ~2 to 9 fold higher than EC20s in the low DOC high hardness CB waters. These observations are consistent with bioavailability models developed for other aquatic species. Results of this research will broaden the geochemical and taxonomic ranges of validation of Ni and Zn bioavailability models.

**48 Overview of EPA's Cooperative Research and Development Agreement with metals associations to develop a simplified metals modeling approach**

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EPA has entered into a Cooperative Research and Development Agreement (CRADA) with eight metals associations in order to leverage scientific expertise and resources of scientists inside and outside of the agency. The CRADA partners and other associated metals experts will collaborate to advance the use of novel scientific approaches for protecting aquatic life from toxic metal exposures. To date, EPA has developed recommended Aquatic Life Criteria for 10 metals (aluminum, arsenic, cadmium, chromium (III and IV), copper, iron, lead, nickel, silver, and zinc). Several of these criteria, however, were developed in the 1980's and do not reflect new toxicity studies or approaches for considering how water chemistry parameters (*e.g.*, pH, dissolved organic carbon, and hardness) can affect metal bioavailability and subsequent toxicity to aquatic species. For example, several recent Aquatic Life Ambient Water Quality Criteria (Freshwater Copper [2007], Draft Saltwater Copper [2016], and Draft Aluminum [2017]) have already been updated to reflect the influence of water chemistry on metal bioavailability using different approaches. This five-year CRADA will support the development of a simplified, overarching modeling approach for quantifying metal bioavailability and toxicity under the range of water chemistry conditions found in aquatic environments. Specifically, this research will include reviewing key modeling parameters and comparing the complexity, accuracy and usability of a variety of possible approaches (*e.g.*, biotic ligand model and multiple linear regression models) to develop a peer-reviewed overarching modeling approach that will reflect a streamlined data approach involving fewer water chemistry input parameters. Towards this objective, methods of model comparison were developed in a recent SETAC Technical workshop (December 2017) that identified best practices for metal bioavailability models.Using the overarching modeling approach as a framework, EPA will then collaborate with individual metals associations to develop metal-specific bioavailability models to support Aquatic Life Ambient Water Quality Criteria development. The modeling approach developed through the collaborative CRADA process will better support states, territories and tribes by providing updated metals criteria recommendations that reflect the most current science and are easier to implement than existing approaches.

## Advances in Passive Sampling Methods: Research to Application

### 49 Statistical comparison of performance reference compound (PRC)-based methods for calculating $C_{free}$

R.M. Burgess, USEPA / Atlantic Ecology Division; A. Joyce, National Research Council/ USEPA / Atlantic Ecology Division

Freely dissolved concentrations ($C_{free}$) have proven useful for estimating exposure concentrations of nonionic organic contaminants in the water column and sediment interstitial waters. It is critical that $C_{free}$ be determined under actual or modelled equilibrium conditions. Performance reference compounds (PRCs) are applied to model equilibrium conditions and estimate fractional equilibrium ($f_{eq}$) used to calculate $C_{free}$. Currently, there are four methods for using PRC data to calculate $f_{eq}$. This variety of methods can create confusion for inexperienced users as to which one to apply. The methods include assuming equilibrium (PRCs are not used), a first order model, a diffusion-based model, and an exchange rate-based model. In this analysis, the four methods were used to calculate $C_{free}$ for several PCB congeners and total PCBs in the water column of a contaminated sediment site. The resulting $C_{free}$ values were then compared statistically to identify trends between the methods. Low-density polyethylene (PE) in four thicknesses was used as a passive sampler and 27 PCB congeners served as the target chemicals. The deployment was performed in triplicate in the water column of three stations at the New Bedford Harbor Superfund site in New Bedford, Massachusetts (USA). Following the 30 day deployments, the four methods were used to estimate $C_{free}$ and these values were then compared statistically with ANOVA followed by Fisher's test. This analysis resulted in twelve comparisons of the multiple PCB congeners and total PCBs. In general, the diffusion-based and exchange rate-based methods resulted in statistically similar estimates of $C_{free}$ while assuming equilibrium often under-estimated $C_{free}$ and using the first order model generated $C_{free}$s statistically-different from the diffusion and exchange rate methods. For example, at the most contaminated site (NBH2), for the 12, 25, 51 and 76 µm PE thicknesses, the diffusion-based and exchange-rate methods $C_{free}$ values were statistically identical 68%, 36%, 68% and 32% of the time, respectively. Based on the comparability of the diffusion method $C_{free}$s to conventional measures of $C_{free}$ from literature studies, this statistical comparison indicates the diffusion-based and exchange rate-based models are the preferred methods to use for calculating accurate water column $C_{free}$s.

### 50 Standardization of Polymeric Sampling for Measuring Freely Dissolved Organic Contaminant Concentrations in Sediment

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Research over the past two decades has demonstrated that the freely dissolved concentrations ($C_{free}$) of hydrophobic chemicals are directly linked to sediment-dwelling organism's exposure to contaminants as well as risk for biouptake into the larger foodweb. Passive sampling methods using polymers can accurately measure $C_{free}$, but historically such measures have been considered research tools largely used by academic researchers. For widespread use in the field and for regulatory decision making, standardized methods are necessary that are accepted by regulatory agencies including the USEPA. The purpose of the present effort is to demonstrate standardized polymeric sampler procedures for measuring $C_{free}$ by multiple participating public- and private-sector analytical laboratories, thereby increasing commercial availability, promoting acceptance, and increased use. This standardization effort, supported by the DoD Environmental Security Technology Certification Program, is progressing through multiple steps of inter-laboratory comparison. In the first step, the laboratories agreed on a common set of performance reference compounds to be used for the measurement of polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs). In the second step, interlaboratory analysis of a common source calibration check standard showed most labs met the acceptance criteria of ±30% for all of the native target analytes and ±50% for PRCs for PAHs and PCBs. A few labs reported results for a few analytes that were slightly out-of-range (e.g. ±38% for one or two natives; ±58% for one or two PRCs). The third step involved the extraction and measurement of PCB and PAH PRCs loaded into polyethylene and polydimethylsiloxane passive samplers and showed most labs met the ±50% acceptance criteria for all the individual PCBs and PAHs. Next steps in the standardization effort involve evaluation of independent passive sampler preparation with loading of PRCs by each laboratory and the use of the samplers in a common homogenized sediment sample to measure $C_{free}$ for the target PAH and PCB compounds. Outcomes of this interlaboratory study are intended to fulfill the method validation requirement for a future SW846 method application.

### 51 Assessing diffusive and advective flux of PAHs across sediment-water and water-air phases at a legacy creosote site in the Pacific Northwest

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This study focused on development of diffusive and advective flux of passive sampler devices (PSDs) at a legacy creosote site in the US PNW. PSDs and proto-type advective flux seepage meters with PSDs were co-deployed at sediment–water and water–air interfaces within and outside the site. PSDs were extracted and analyzed by gas chromatography mass spectrometry. Analyses included four instrumental methods encompassing parent and alkylated polycyclic aromatic hydrocarbons (PAHs), parent and alkylated PAHs specific to forensic source determination, oxygenated PAHs, and hopanes/cholestanes. Preliminary results show PAHs were detected in all compartments across all sites, with highest concentrations downstream. Compounds included phenanthrene and benzo[b]fluoranthene, currently found on the EPA Priority PAHs list. Concentrations of phenanthrene were 27.8 ng/L/80.3 ng/L in deep/shallow porewater, 5.63 ng/L in surface water and 44 ng/m3 in air downstream. We report each environmental compartment concentration, and the bulk movement of contaminants through the environment, referred to as flux. The diffusive flux of air, water and sediment has only recently been demonstrated for the first time with low density polyethylene passive samplers. Further data analysis will inform the direction and magnitude of diffusive PAH movement between the sediment, aqueous, and atmospheric phases at this heavily contaminated site. Additionally, we will report on the results of proto-type advective flux meters and co-deployed PSDs. Advective flux describes bulk movement of water and its contribution to the transport of contaminants, allowing us to take into effect factors such as tidal pumping, not accounted for in a static diffusive flux model. Combining these two components will allow us to better understand fate and transport of environmental contaminants. Forensic source identification determines contributions from pyrogenic and petrogenic sources, to provide insight into the potential impact of the facility on the surrounding area. Hopanes/cholestanes serve as biomarkers of petroleum products, and also help in sourcing multiple types of crude oil. OPAHs are photodegredate compounds of PAHs, have been understudied and can be more toxic than their PAH counterparts. Understanding contaminant movement across environmental phases at contaminated sites will help to inform managers and regulatory agencies responsible for the maintenance and decontamination of these locations.

### 52 Passive Porewater Sampling: From Academia to Consulting

*<u>L. Paulik</u>, Maul Foster & Alongi, Inc. / Environmental and Molecular Toxicology; M. Pickering, Maul Foster & Alongi, Inc.*

The research community has long accepted passive sampling as a useful tool for accurately measuring concentrations of organic contaminants in sediment porewater. However, obtaining regulatory approval to use these newer passive techniques can be challenging. In more recent years the regulatory community in the United States has been increasingly accepting and implementing passive sampling. This presentation will focus on an ongoing project that takes place in a freshwater pond in the Columbia Slough Watershed in Portland, Oregon. The pond provides habitat for sensitive native species, but it is contaminated with legacy PCB pollution. The site's property owner is working with the Oregon Department of Environmental Quality (DEQ) and Maul, Foster & Alongi (MFA) to prepare the remedial design for the pond. MFA developed a porewater passive sampling plan to establish baseline conditions and monitor the effectiveness of the remedy. This presentation will discuss challenges and lessons learned during development of the passive porewater sampling approach for this project. Care was taken to ensure that the approach would satisfy the guidelines outlined in "Laboratory, Field, and Analytical Procedures for Using Passive Sampling in the Evaluation of Contaminated Sediments: User's Manual", a guidance document on passive porewater sampling authored by the U.S. Department of Defense's Strategic Environmental Research and Development Program/ Environmental Security Technology Certification Program and USEPA's Office of Research and Development in 2017. MFA is planning to use SiREM's SP3 sampler to passively sample porewater before and after remedy implementation. One source of uncertainty with any passive sampling technique is the calculations used to convert concentrations in passive samplers to environmental concentrations. A major consideration in choosing a passive sampling approach was whether environmental data collected now would be directly comparable to data collected years in the future. SiREM provides users with both raw data from the passive samplers and environmental concentrations. This gave MFA's scientists confidence that the baseline data will be directly comparable to all future data collected with this SP3 sampler at this site, regardless of advances in passive sampling research.

### 53 Utilization of a novel passive sampler to detect PFASs in aquatic environments

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Poly- and perfluoroalkyl substances (PFASs) are of growing concern worldwide, due to their ubiquitous presence and adverse health effects in humans and the environment. Surface waters in the northeastern United States in particular have displayed elevated concentrations of PFASs. Here we utilize microporous polyethylene (PE) tube passive samplers to gain a better understanding of the sources and spread of these contaminants. Two sampling campaigns were conducted in the fall of 2017 and summer 2018, deploying a total of seventy-two PE tube passive samplers (containing Hydrophilic-Lipophilic-Balanced sorbent) across nine sites in Narragansett Bay (RI, USA) and two wastewater treatment plant effluents. 25 PFAS compounds were measured across all sites in the passive samplers, as well as analogous water and sediment samples. Initial results suggest passive sampler concentrations ranging from 0.05-6.5 ng sampler$^{-1}$ day$^{-1}$, with sampling rates of 40 to 70 mL day$^{-1}$. Results also indicate that the PE tube samplers take up PFASs in a similar ratio as observed in water samples. This preliminary study shows strong potential for the use of the PE tube sampler for PFAS detection in aquatic environments. By using passive samplers to analyze the spatial and temporal trends of these contaminants we can better assess their longevity in water and connected environmental compartments.

### 54 Using passive samplers and biomonitoring organisms to monitor polycyclic aromatic compounds and naphthenic acids in boreal wetlands

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Several recent studies have reported evidence that surface mining operations in northern Alberta's oil sands region contribute significantly to the deposition and accumulation of inorganic (metals) and organic contaminants (polycyclic aromatic compounds (PACs) and naphthenic acids) within the vicinity of major bitumen upgrading facilities and oil sands developments. As part of the Canada-Alberta Joint Oil Sands Monitoring Program, we are characterizing the risk of exposure of wildlife, including amphibians, to these contaminants of concern. In addition to collecting abiotic (water and sediment) and biotic samples (wood frog tadpoles, *Lithobates sylvaticus*), we have deployed an array of passive sampling devices to complement these more traditional sampling techniques. In the spring of 2017, over the course of the amphibian breeding season, we equipped boreal wetlands (n=12) situated at varying distance from oil sands mines with polyurethane foam (PUF) samplers and semipermeable membrane devices (SPMDs) to measure PACs in air and water, respectively, and with polar organic chemical integrative samplers (POCIS) to measure naphthenic acids. In general, passive sampling devices accumulated a greater degree of organic contaminants in wetlands north of Fort McMurray, Alberta that were located inside the mineable oil sands area. The data collected helps us explore the use of passive samplers to identify potential contaminant 'hot spots' and conduct spatial assessments of contaminant accumulation in northern Alberta wetlands. Further, the contaminants data generated from our passive sampler work helps us address whether specific wetland monitoring sites accumulate contaminants through atmospheric deposition versus hydrological inputs, and explore correlations between contaminants accumulating in samplers with those bioaccumulating in wood frog tadpoles. In addition, we can use extracts from the organic contaminants accumulated by the SPMDs to characterize the toxicity of PAC mixtures using sensitive in vitro bioassays.

### 55 Considerations for applying experimentally-derived sampling rates to calculate organic contaminant concentrations in water from POCIS data

*<u>D.A. Alvarez</u>, USGS-CERC / Environmental Chemistry*

The global use of the polar organic chemical integrative sampler (POCIS) to monitor waters for organic contaminants with moderate to high hydrophilicity has grown rapidly over the last decade. Their use in monitoring studies has far outpaced the research to understand the sampling kinetics which is critical to obtaining reliable estimates of water concentrations. Passive sampling devices have many uses and applications; however, the ultimate goal for any type of sampler is to accurately estimate the ambient concentrations of target analytes. For integrative samplers such as the POCIS, this requires knowledge of the chemical-specific sampling rate. Sampling rates in water have been experimentally determined for over 350 individual chemicals with pesticides and pharmaceuticals accounting for approximately 75% of these chemicals. In this presentation, the role of environmental factors such as water flow, temperature, salinity, and biofouling on the uptake of chemicals will be discussed along with the applicability of the performance reference compound (PRC) approach. The proper selection of sampling rates from the literature is key in increasing the accuracy of estimating a chemical's time-weighted average concentration in water. Considerations for how a sampling rate was determined, its comparability to a specific study, and how the estimated chemical concentrations in water may be affected will also be discussed.

**56 Passive samplers of polar compounds: Application (front-end) and science (back-end)**

*K. Booij, PaSOC*

The use of adsorption based passive samplers of polar organic compounds is still largely an academic endeavor. Their use in applied and regulatory monitoring is hindered by incomplete understanding of the mechanistic basis of these samplers. As a result it is difficult to assign uncertainty estimates to passive sampling results and to take the effects of flow velocity, temperature, and biofouling properly into account. Limited understanding also makes it difficult to assess the quality of reported sampling rates, which can be rather divergent, even for the same compound and the same sampler design. Accumulation of target compounds includes transport through the water boundary layer, the biofouling layer (if present), the membrane, and finally into the sorbent. Each of these phases can have an effect on the sampling rate. The series resistance model will be used to illustrate how the importance of each of the transport steps can be separately assessed. This allows to evaluate if the overall sampling rate is quantitatively understood, including the effects of flow, temperature, and biofouling, and including the uncertainties that are associated with these effects. If sampling rates are sufficiently understood, the model can be programmed into a user friendly calculation template for end users. If not, wider uncertainty levels need to be applied. A scheme that identifies the different roles of sampler developers (science) and end users (application) will be presented. The role of sampler developers is to establish relationships between sampling rates and flow, temperature, and biofouling, and to program this into a modeling template. End users are responsible for the chemical analysis and the measurement/estimation of temperatures, flow velocities, and biofouling, including the uncertainties in these estimates, and to feed these data into the template. The template then gives an estimate of aqueous concentrations and an uncertainty estimate of this value. The models are available. The methods are available. We can reach out to end users and make this work.

## Teaching Environmental Chemistry: Where Are We and Where Are We Going?

**57 Holistic Faculty Development for Environmental Chemistry Faculty**

*C.M. Lee, Clemson University / Environmental Engineering and Earth Sciences Department; K.A. High, Clemson University / Engineering and Science Education*

Faculty who specialize in science, technology, engineering, and mathematics (STEM) experience a variety of demands in the 21$^{st}$ century. Expectations for teaching, research, service, and leadership have increased at top research universities as well at predominantly undergraduate institutions. Traditionally faculty development programs in higher education focus on teaching and learning. But many studies in STEM education research have pointed to the lack of progress in evidence-based teaching practices being widely adopted. Environmental chemistry faculty who are engaged in either undergraduate or graduate research must often obtain funding, hire and supervise students, and maintain instrumentation and other lab equipment while also meeting teaching and service responsibilities. Our hypothesis is that increased expectations for research productivity, service responsibilities, and leadership opportunities have constrained adoption of evidence-based practices. We will present NSF-sponsored research that support our hypothesis. Results from a literature review, a national workshop conducted with STEM faculty, and interviews suggest that approaches to faculty development that considers all aspects of faculty careers will enhance teaching and learning as well as improve faculty well-being.

**58 A Community-Based Research Approach to Teaching Ecotoxicology: Building Student Competencies in Science and Civic Engagement**

*S.R. Tuberty, Appalachian State University / Biology*

In the last few decades there has been a wealth of expert evidence-based research published on the links between new pedagogy and learning, as well as psychological and cognitive ability and limitations that can inform faculty on what teaching techniques create the most skill development and measurable learning outcomes for our students. In 2008, the National Survey of Student Engagement (NSSE) published a now-familiar list of what is referred to as 'high-impact practices.' These are the college experiences that highly correlate to the most powerful learning outcomes. Among the list of ~10 practices are undergraduate research, collaborative projects, service learning & community-based learning, and capstone courses and projects. These are called 'high-impact practices' because participation in them correlates with high retention and persistence rates and because they induce student behaviors that lead to meaningful learning gains. The important student behaviors include the following: 1) Investing time and effort; 2) Interacting with faculty and peers about substantive matters; 3) Experiencing diversity; 4) Responding to more frequent feedback; 5) Reflecting and integrating learning; and 6) Discovering relevance of learning through real-world applications. Over the last ten years I have created and implemented a dual-listed capstone Ecotoxicology course (senior undergraduate/M.S. level grad) that leverages several of these 'high-impact practices.' From a combination of lectures, skill & technique focused labs (supported with external grants), experiential learning techniques, and semester-long community-based group research projects, students develop knowledge and skills related to toxicology and other STEM area careers that benefit from these transferable skill sets (e.g., pharmaceutical, sustainable development and practices, and biotechnology careers). In addition to these important outcomes, the community partners add outside the classroom real-world needs and expectations that increase student 'buy-in' and opportunity for students to increase their knowledge of civic engagement and learn about related careers in their communities and beyond.

**59 Translating Environmental Chemistry Research from the Undergraduate Lab to the High School Classroom: Operation Precipitation Collaboration**

*J. Faust, K. Miller, K. Wokosin, The College of Wooster / Chemistry; W. Breneman, Smithville High School*

The primary removal mechanism of organic matter from the atmosphere is wet deposition, which transports pollutants, alters the global nitrogen budget, and impacts human health. Furthermore, precipitation offers clues to the composition of long-lived cloud droplets, which affect global climate by scattering or absorbing incoming solar radiation. A precipitation collection program in northeast Ohio was developed in collaboration with a local high school to monitor organic contaminants in rainwater while also introducing high school students to environmental chemistry research. From fall 2017 through spring 2018, juniors in the honors-level biology course at Smithville High School learned how to prepare field blanks, avoid sample contamination, and keep detailed notes. Meanwhile, undergraduate researchers at the College of Wooster analyzed the rainwater samples with solid phase extraction (SPE) and gas chromatography-mass spectrometry (GC-MS). Notably, the pesticide metolachlor was identified in rainwater collected at all sites. At the end of the school year, an undergraduate researcher involved in the project returned to the high school to explain the analysis techniques, findings, and implications. Plans are underway to continue the collection program in the 2018-2019 academic year.

**60 Fostering environmental chemistry education through incorporation with aquatic ecology**

*S. Nutile, Penn State Univ., The Behrend College / Biology*

As aquatic environments serve as the final repository for many anthropogenically derived contaminants, the study of aquatic ecology is largely dependent upon understanding environmental science and chemistry. Abiotic factors influence contaminant exposure and response of biota existing in aquatic ecosystems, thus influencing resulting aquatic communities. As aquatic ecology largely focuses on evaluating the health, history, and future of aquatic environments through sampling macroinvertebrate communities, aquatic plants, and measuring various water quality parameters, macroinvertebrate densities, species richness and diversity, chlorophyll a, water clarity, and nutrient levels all depend heavily of current and historical land-use patterns, runoff, and contamination. Without a full complement of sampling of these various parameters and understanding of the role of environmental chemistry in dictating and influencing these measured parameters, full assessment of the health and future of aquatic habitats is not possible. As such, environmental chemistry plays an undeniable role in developing a comprehensive understanding of aquatic ecology and educators teaching these courses have an opportunity to create truly integrative course for students; highlighting the diversity of environmental science and the importance of a broad science curriculum. In the fall of 2018, undergraduate students at Penn State Erie will engage in an Aquatic Ecology course with a laboratory component designed to integrate aspects of ecology, environmental chemistry, and biology. The laboratory portion of this course will challenge students to evaluate two field sites using traditional aquatic ecology sampling methodologies, environmental analysis of contaminants, water quality parameters, and historic land-use data to generate a report assessing the current health of these systems in comparison to one another and identify the major drivers that have led to this evaluation. Ideally, this laboratory structure will deepen students understanding of topics covered in lecture and appreciation of the role of environmental chemistry and science within the biology curriculum, as well as provide evidence for educators to improve educational diversity within scientific courses. This presentation will focus on the success of this approach to date, lessons learned, student perception of the value of such an exercise, and suggested improvements on this approach for the future.

**61 Using models to integrate chemistry and biology in environmental science and engineering.**

*C.A. Ng, University of Pittsburgh / Civil & Environmental Engineering*

A persistent challenge in STEM education is the training of undergraduates in design thinking. This requires the integration of conceptual knowledge to solve ill-defined or open-ended problems typical of real-world science and engineering applications. In environmental engineering and science, challenges are intensified by the interdisciplinary depth required to solve environmental challenges—students must successfully integrate concepts from biology, chemistry, and physics, among other fields. Modeling and simulation have the potential to address these challenges, while engaging students and improving their perception of their own knowledge and skills. Through the use, revision, and construction of models, students can go beyond "rote memorization" to engage critical thinking about both the concepts involved and the students' understanding of the concepts. When model construction is done collaboratively and reflectively the process allows students to probe, revise, and refine their knowledge about objects, concepts, and complex phenomena. Here, I will discuss a series of models and modeling activities I have developed at the University of Pittsburgh for both undergraduate education and K-12 outreach and their use to improve student engagement and retention in STEM. The first is a curricular module designed for middle and high school students, which uses agent-based modeling in NetLogo to engage students in understanding chemical fate and toxic impacts in aquatic ecosystems. The model is easily extendable and can also be used to engage high school seniors and college students in "low-stakes coding" to develop their own informative models. The second and third are modeling exercises aimed at sophomore- and senior-level undergraduates in environmental engineering, and are aimed at preparing them to solve open-ended problems in Senior Design. It is hoped that this presentation will spark discussions and conversations that can lead to model development and model exchanges across diverse curricula where environmental chemistry has a role.

**62 Project-Based Learning Initiatives in Environmental Chemistry**

*J.C. D'eon, S. Joudan, University of Toronto / Chemistry; J. Faust, The College of Wooster / Chemistry; S. Baskaran, University of Toronto – Scarborough / Chemistry; S. Kavassalis, University of Toronto / Chemistry*

Over the past five years project-based learning initiatives have been implemented in several environmental chemistry classes at the University of Toronto. These projects span the University curriculum; from a second-year project that places students in the role of a science advisor to a delegation attending the 1987 meeting in Montreal, which resulted in the Montreal Protocol that controls the emission of ozone depleting chemicals; to a fourth-year lab-based project where students carry out their own environmental analysis from hypothesis to lab work to presentation and report. These projects will be discussed in terms of student learning gains and a potential increase in student engagement. The more intricate details of environmental science, such as climate data and air quality measurements, are best told using visualizations such as graphs and figures. This presentation will also describe how these environmental chemistry projects have been tailored to teach students how to read and create visualizations.

**63 Establishing a long-term monitoring program of a perfluoroalkyl acid contaminated hotspot using a senior analytical environmental laboratory course**

*S. Joudan, University of Toronto / Chemistry; A.O. De Silva, Environment and Climate Change Canada / Aquatic Contaminants Research Division; S. de Solla, Environment Canada / Ecotoxicology and Wildlife Health Division; J.C. D'eon, University of Toronto / Chemistry*

Per- and polyfluoroalkyl substances are a class of ubiquitous environmental contaminants that are used in many products and applications, including as an active component in aqueous film forming foams, which are used to extinguish fuel fires. Many contamination hotspots have been reported throughout North America and worldwide, often associated with military training sites and airports. In 2012, a perfluoroalkyl hotspot was identified in Southern Ontario, a one-hour drive from the University of Toronto. Contamination at this location was traced back to firefighter training activities at the John C. Munro International Airport. This site provides us with a unique opportunity to visit a contaminated site with our fourth-year analytical environmental chemistry course and monitor its recovery over time. We visited the site in the fall semesters of 2016 and 2017, along with a nearby background site, to collect water, sediment, invertebrates and fish samples. Over two laboratory periods students performed matrix-specific extractions and analyzed the samples for 11 perfluoroalkyl acids (PFAAs) using liquid chromatograph tandem mass spectrometry. Data was shared amongst the class. In addition to the skills of sample collection, extraction, and analysis, the learning objective of this experiment was to guide students through the process of data analysis and effective communication of results through figures and graphs. This process was facilitated by a report outline that included discussion points and relevant figures. Students received detailed feedback on their outlines related to their choice of data presentation and its effectiveness in conveying their discussion points. Most students were able to find comparisons between concentrations of the contaminants, such as the relationship between carbon chain length and environmental partitioning. This experience provided students with tools for data analysis and visualization that could be applied in the course and their future research. Overall, students gained real sampling experience, and were able to compare their results to those published in peer review journals and from previous classmates, which better represented the reality of environmental monitoring. As there are many other contaminated sites around the world, we believe this experience can be applied at many other institutions.

**64 Mentoring students in a research laboratory at an undergraduate-focused institution**

*J.K. Challis, University of Manitoba / Chemistry*

The University of Winnipeg (UW), Manitoba, Canada is predominately an undergraduate institution, often praised for its teaching, small class sizes, and accessibility; aspects of a post-secondary education sometimes lost in larger research-focused universities. However, the UW also has a strong, under-the-radar research identity that relies heavily on undergraduate students through summer research positions, directed-study credits, Honours students, and Co-op programs. This presentation will summarize my experiences training and mentoring undergraduate students in an environmental analytical chemistry laboratory. Given the short-term research stints typical for many undergraduate students, training must be efficient and study design needs to be both tractable over a summer and scalable for future students. Our approach to training and mentorship will be discussed, drawing on projects as case-studies to highlight successes, challenges, and suggestions moving forward.

## Immunotoxicology: Identifying Adverse Effects, Developing New Approaches and Confronting Existing Challenges

**65 Examining the acute immunotoxicity of oil sands process-affected water using mammalian macrophages**

*L. Fu, C. Li, D. Lillico, N. Phillips, University of Alberta / Biological Sciences; M. Gamal El-Din, University of Alberta / Civil and Environmental Engineering; M. Belosevic, J. Stafford, University of Alberta / Biological Sciences*

Research examining OSPW toxicity has focused on the naphthenic acid (NA) containing organic fraction (OSPW-OF), indicating it as the primary toxic component. In this study we used an array of in vitro cell-based assays to explore OSPW toxicity and to directly compare the toxic effects induced by whole OSPW and OSPW-OF based on their relative NA content. By examining the viability, gene expression, and functional activities of mouse macrophages, we observed that the whole OSPW was significantly more toxic at NA concentrations ranging from 10-18 mg/L, compared to OSPW-OF. Specifically, our results showed that whole OSPW had a dramatic effect on the proliferation of mammalian cells, and at the highest doses tested (i.e., 12-18 mg/L) it significantly reduced cell viability and promoted cell lysis. This caused a reduction in the total cell numbers, which was not observed after the cells were exposed to equivalent doses of OSPW-OF. At the dose of 10 mg/L NAs, whole OSPW did not significantly affect viable cell numbers but the monitoring of selected stress genes indicated that a possible mode of action for OSPW-induced toxicity includes oxidative stress and associated DNA damage. We also measured pro-inflammatory cytokine expression levels after OSPW exposures to provide important information regarding its effects on mammalian immunity. Our results showed that whole OSPW at 10 mg/L NAs exhibited differential effects on basal cytokine gene expression and cytokine secretion in resting cells. In addition, whole OSPW had variable influences on the mRNA expression and protein secretion levels in activated cells. Alternatively, exposure to the OSPW-OF at 10 mg/L NAs did not induce significant effects on basal or activated cytokine gene expression or secretion. These data suggest the presence of immunomodulatory factors within whole OSPW (possibly inorganic constituents) that selectively affect cytokine networks in immune cells. The complexity of these effects requires further investigation but our results also indicate that whole OSPW-exposed macrophages have a reduced ability to engulf bacteria, which may also translate in vivo to reduced host defense capability. Overall, these observations suggest that the immunomodulatory effects of as yet unidentified OSPW constituents are dose-dependent and they can differentially affect the cell biology of immune cells at the gene, protein, and functional levels.

**66 Evaluating Immune Competence and Chemical-Induced Immunotoxicity in Amphibians**

*M. Gallant, University of Saskatchewan / Toxicology Centre; N.S. Hogan, University of Saskatchewan / Department of Animal and Poultry Science and Toxicology Centre*

Numerous chemicals in the aquatic environment are known to modulate immune parameters in fish and amphibians, reducing their ability to resist pathogen infection and increasing incidence of disease. This is particularly relevant for amphibians as global amphibian declines have often been attributed to disease. However, there are few well-established endpoints for immune competence assessment in amphibians, which limits studies on the functional effects of chemicals exposure to pathogen susceptibility. The aim of this research was to develop an amphibian model to that would enable us to explore the possible mechanisms of the immunotoxic effects and to evaluate amphibian immune competence in an activated immune system. We first tested a blood cell staining method that enabled leucocyte counting and partial differentiation by flow cytometry. Blood was taken from juvenile *Xenopus laevis* and incubated with the lipophilic dye, 3,3'-dipentyloxacarbocyanine iodide (DiOC5(3)). Distinct populations of lymphocytes, granulocytes and monocytes were identified by flow cytometry and the number of lymphocytes and granulocytes counted using this method was in good agreement with those counted microscopically. We then tested concentrations of lipopolysaccharide (LPS), a potent immune stimulant, that could be injected to modulate white blood cells populations and cytokine expression. Intraperitoneal injection of 0.3-3 ug/g LPS resulted in a dose-dependent decrease in lymphocyte population and increase in granulocyte and monocytes as well as an induction of inflammatory cytokine expression in the kidney. Finally, these methods will be used in a host resistance assay to determine whether short-term exposure to a known immunotoxicant, benzo[a]pyrene (B[a]P), modulates immunologic responses to an LPS-challenge. We anticipate that this work will help further assessment of immune competence in amphibians and will provide information on the immunomodulatory effects of B[a]P (and eventually other chemicals) on amphibian immunity.

**67 Proposed minimum requirements for the immunotoxicity test to assess chemically induced alteration of susceptibility to pathogen in fish**

*K. Nakayama, S. Kitamura, Ehime University / Center for Marine Environmental Studies*

Recently, alteration of susceptibility to pathogen has been selected as an endpoint to evaluate the immunotoxicity of environmental contaminants in fish. It is thought as an ideal method, since chemically-induced immunosuppression ultimately leads to spread and/or outbreak of infectious disease. For the test, several different fish species have been used as host animal, and of course, pathogen used in the experiment depends on the host fish. Therefore, the experimental methods varied among studies due to the difficulty in standardizing the test method. Because of the absence of a standardized method, the experimental infections in some studies were conducted in an inappropriate way. To avoid this and misunderstanding the results of infection test, we would like to propose minimum requirements for the immunotoxicity test based on our experience. We have been using common carp (*Cyprinus carpio*) as a host fish, and conducted the infection tests with six bacterial species to determine which species are suitable for our experiment. Among them, three species including *Aeromonas hydrophila*, *A. veronii*and *Edwardsiella tarda* caused mortality in carp, however, mortality was observed only at one- or two-day post-infection, and the survived fish were not dead by the end of experiments. Mortality observed in these experiments was probably caused by toxins produced by the infected bacteria. In these cases, even if the infected fish were exposed to a test chemical, its immunosuppressive effect are not evaluated properly. Therefore, it is better to present the time-course of mortality of host fish, which represents how the fish were dead, by toxins or by immunotoxicity. Additionally, the detection of pathogen from host is also preferred to indicate that a test compound has an immunosuppressive potential. Either reisolation of pathogen using a selective medium or (quantitative) PCR can be applicable. Our improved test method with the combination of common carp and *A. salmonicida* will be presented.

**68 The arylhydrocarbon receptor (AhR) – a mediator of immunotoxicity in fish? AhR-signaling in immune and liver cells of rainbow trout.**

*A. Moeller, University of Bern / Centre for Fish and Wildlife Health; J. Song, Ehime University / Centre for Marine Environmental Studies; A. Nakayama, University of Bern / Centre for Fish and Wildlife Health; K. Nakayama, S. Kitamura, Ehime University / Center for Marine Environmental Studies; H. Segner, University of Bern / Centre for Fish and Wildlife Health*

The ligand-activated transcription factor aryl hydrocarbon receptor (AhR) senses small chemical molecules including important environmental contaminants, such as dioxins, polychlorinated biphenyls and polyaromatic hydrocarbons. Hallmarks of AhR-mediated toxicity in vertebrates are hepatotoxicity and immunosuppression. More recent studies have shown that the AhR plays an important role in the regulation of the mammalian immune system. For teleost fish, it is known that AhR-activating xenobiotics have immunosuppressive effects, but the presence and role of the AhR in the fish immune system has not been explored yet. Here, we demonstrate that immune organs and cells of rainbow trout, *Oncorhynchus mykiss*, express transcripts of the AhR isoforms, AhR2α and AhR2β. At levels comparable to transcript levels in the trout liver. Exposure of isolated trout immune and liver cells to equipotent concentrations of the AhR ligand, benzo(a)pyrene (BaP) resulted in cell-specific gene transcription profiles. Global gene expression analysis revealed that in the liver cells BaP regulated mainly genes involved in transcription and biosynthesis processes. In contrast, in the immune cells, particularly immune-related genes were regulated by BaP exposure. Genes that were commonly regulated in both cell types included CYP1A1, CYP1A3 and sulfotransferase. The findings suggest an immunoregulatory function of AhR in teleostean fish.

**69 Conserved & Differential Transcriptional Immune Responses In Commercially & Recreationally Important Fish Species Exposed to Oil, a Pathogen, or Both**

*M.L. Rodgers, The University of Southern Mississippi / Division of Coastal Sciences; M.S. Sepulveda, Purdue University / Department of Forestry and Natural Resources; R.J. Griffitt, University of Southern Mississippi / Coastal Sciences*

Polycyclic aromatic hydrocarbons are known to produce immunotoxic effects in fish and other organisms. There are relatively few studies that directly compare immune transcriptomic responses across multiple fish species to determine conserved immune responses (both in terms of gene expression and pathway regulation) to toxicants and other stressors. Evidence for the impacts of polycyclic aromatic hydrocarbons (PAHs) on immune function in fish is contradictory, with PAH type, concentration, route of exposure, and possibly even fish species driving the differing effects. In order to further understand the conserved and differential immune responses to PAHs in various fish species, three commercially and recreationally important fish species in the Gulf of Mexico were used: juvenile red snapper (*Lutjanus campechanus*), Atlantic croaker (*Micropogonias undulatus*), and red drum (*Sciaenops ocellatus*). All three species (snapper, croaker, and drum) were exposed to high energy water accommodated fractions of oil for 7 days, then exposed to the known fish pathogen V. anguillarum for one hour, followed by an additional 24 hours of oil exposure (8 days total). Upon completion of the experiment, spleens were removed from all fish and RNAseq and pathway analyses were performed to examine regulation of immune-related genes and their networks. Previous research in our lab with southern flounder (*Paralichthys lethostigma*) using a similar experimental approach found that oil alone caused suppression of immune-related genes such as irf8 and gata3 in the liver, and that exposure to V. anguillarum alone induced a different suite of immune genes, with upregulation generally occurring rather than suppression. In addition, previous research in our lab on adult sheepshead minnows (*Cyprinodon variegatus*) revealed that exposure to oil impacted innate immune pathways (for example, those related to macrophage function), and to a lesser extent adaptive immune pathways (for example, those related to B cell function). However, the flounder and sheepshead minnow RNAseq was performed on liver samples. The spleens of the three species herein present a unique opportunity to focus on an immune organ to fully understand the impacts that oil, a pathogen, or both together, have on immune genes and pathways in fish, and how these genes and pathways are either conserved or differ from one another.

**70 Immunomodulation in adult largemouth bass (*Micropterus salmoides*) following seasonal exposure to environmentally relevant contaminants and mixtures**

*L.R. Iwanowicz, United States Geological Survey / Leetown Science Center; J.K. Leet, C.A. Richter, R.W. Gale, D.E. Tillitt, US Geological Survey / Columbia Environmental Research Center*

Disease outbreaks, skin lesions, fish kill events, and reproductive abnormalities have been observed in wild populations of Centrarchids in the Chesapeake Bay watershed. Occurrence of synthetic and natural hormones from wastewater treatment plants and livestock operations, pesticides from agricultural lands, and phytoestrogens have been implicated as potential causes of these adverse effects. Our objective was to investigate possible immunomodulation in adult largemouth bass (*Micropterus salmoides*) in response to a seasonal exposure to environmentally relevant contaminants in outdoor mesocosms. Exposures included 17alpha-ethinylestradiol (EE2; 2.4 ng/L) or a binary mixture of endocrine-active substances commonly detected in the Chesapeake Bay watershed, atrazine (5.4 μg/L) and estrone (47.9 ng/L). The 5-month exposure was conducted from July to December. Functional immune responses of anterior kidney derived leukocytes were evaluated in December at the end of the exposure period, and the following April just prior to spawning. Concentrations of EE2 and estrone in the mesocosms fell below detectable levels in December, but detectable concentrations of atrazine (2.3 μg/L) persisted at least through March. For each sampling time, anterior kidney leukocytes were isolated and grown in primary culture for the assessment of zymosan-stimulated respiratory burst and lectin-stimulated mitogenic responses. Binary mixture exposure (atrazine and estrone) led to differences in respiratory burst stimulation over time and treatment with a significantly greater response in April relative to December (Mann-Whitney; $P < 0.001$). Respiratory burst activity was significantly greater in April relative to control fish assayed in April (Kruskal-Wallis One Way on Ranks, Dunn's post hoc vs control; $P = 0.008$) for this treatment as well. There were no significant differences in responses between sexes. We observed a significantly dampened mitogenic response to PHAP (a T cell mitogen) and LPS (a B cell mitogen) in the EE2 treatment relative to control fish sampled in April. Our results demonstrate that environmentally relevant concentrations of contaminants can alter immune function in an economically important fish species.

**71 The Sexually Dimorphic Immune System: An Exploration of the Influence of Sex and Sexual Maturity Status on Immune Function and Immunotoxicity in Fish**

*M.K. Sellin Jeffries, Texas Christian University / Department of Biology*

A wide variety of abiotic and biotic factors are known to influence immune function and disease resistance. In mammals and birds, it has been well established that organism sex and sexually maturity status can influence various aspects of immunity; however, less information regarding the influence of these parameters on immunity is available for fish. This talk will provide an overview of several studies, conducted in fathead minnows (a common ecotoxicological model), that demonstrate the sexually dimorphic nature of the immune system. These studies have noted several key differences in immune function between male and female fathead minnows and between more and less sexually mature male fathead minnows. For example, studies have repeatedly shown that male fathead minnows are less able to survive infection with *Yersina ruckeri* (the bacteria responsible for enteric red mouth disease) than their female counterparts. Similarly, evidence suggests that male minnows with more prominent secondary sexual characteristics (an indicator of maturity status) are less able to resist infection than those with less prominent characteristics. The data demonstrating such differences will be outlined in detail, along with the physiological underpinnings responsible for such differences. In addition, data showing that the male and female immune systems respond differently to contaminant exposures will be discussed in an effort to demonstrate the importance of considering the sexual dimorphic nature of the immune system in the design and interpretation of immunotoxicity studies.

**72 Understanding molecular mechanisms of action in immunotoxicology for ecological risk assessment through the use of adverse outcome pathway analysis**

*C.D. Rice, Clemson University / Biological Sciences; M.L. Rodgers, The University of Southern Mississippi / Division of Coastal Sciences*

Considerable evidence demonstrates the adverse effect of immune-compromise, whether stemming from inherited primary immunodeficiency, or acquired secondary immunodeficiency, such as chemical induced immunotoxicity or disrupted neuroendocrine-immune interactions leading to stress. The possible adverse outcome from these immune dysfunction(s) is increased susceptibility to pathogens, and possibly death, or even chronic inflammation. From this point, one can intuit the effects of reduced survival in terms of local population size, and the resulting negative impact on community structure and function – not to mention the possible spread of pathogens throughout the ecological system. This thought process should, in principle, be applicable to adverse outcome analysis (AOA) for use in determining the ecological risk associated with immunodeficiency as a result of exposure to contaminants. However, unlike endocrine disruption AOA for reproductive toxicants, in which the basic tenants of reproductive anatomy and physiology (A&P) are fairly consistent across fish taxa, the immune system A&P can vary. As an example, the A&P of killifish, rainbow trout, and cod immune systems are different. In addition, unlike other physiological systems, parenchymal cells of the immune system continually traffic from primary lymphoid organs throughout the organism in search of pathogens concentrated within the secondary lymphoid organs. Thus, immunocompetent cells may have a different exposure history to contaminants compared to localized cells of other physiological systems. For ecological risk assessment using AOA, any possible ecological effect of immune-compromise needs to be linked to one or more molecular initiating events (MIE), and one or more subsequent key events. Currently, we know more about the key events following exposure to immunotoxicants than the MIE. Therefore, we need to identify MIE specific to immune function(s) that could be consistently applied to specific individual, classes, or combination of contaminants. The question remains – is this possible? Moving forward, the aquatic immunotoxicology community needs to take a more collaboratively focused approach, and deep genomics to identify candidate genes targeted by MIE will no doubt be important. For environmental immunotoxicology to have its place in ecological risk assessment, focused workshops on how to incorporate AOA are needed at both the National and International level.

## Per- and Polyfluoroalkyl Substances: Recent Advances and Future Directions – Part 1

**73 Qualitative and quantitative screening of the bioaccumulative potential of PFASs in aqueous film forming foam using human serum albumin**

*W. Li, University of California Davis; T.M. Young, H.N. Bischel, University of California Davis / Civil and Environmental Engineering*

Aqueous film forming foams (AFFF) developed in the 1960s by the United States Navy and the 3M Company have been widely used and contain poly- and perfluoroalkyl substances (PFASs). Although multiple C8-PFASs (e.g., PFOS and PFOA) have been phased out of production due to toxicity, persistence in the environment and bioaccumulative potential, PFASs with similar structures such as 8:2 FTOH continue to serve industrial needs. Due to the large number of commercially available and environmentally detected PFASs, the bioaccumulative potential of PFASs used to replace legacy structures is difficult to assess. Further, the current EPA reference method for PFAS detection was designed based on a limited number of PFASs. In this study, a comprehensive PFAS screening library and HPLC-Qtof-MS method was developed to qualify and quantify PFASs in AFFF. The blood protein, human serum albumin (HSA), was then exposed to a series of AFFF dilutions using equilibrium dialysis to screen for preferential associations of specific PFASs with the model protein. A broad spectrum of PFASs, including C4-PFASs, identified in AFFF preferentially associated with HSA, suggesting a potential for bioaccumulation. Binding was not linearly correlated with the concentration of PFASs in AFFF, further implying the possibility of high binding affinity at specific sites. Preliminary results indicated that after precipitation of the protein and removal of all free PFASs in solution, the hydrolyzed HSA pellet contained strongly bound PFOS and PFHpS. Further analysis using $^{19}$F NMR and molecular docking tools is underway to evaluate mechanisms of observed protein-ligand interactions and the potential for covalent binding of PFAS with HSA. The combination of experimental and modeling techniques applied is expected to provide value in rapidly assessing the bioaccumulative potential of emerging PFASs in commercial products.

**74 Immunotoxicological Findings of an Aqueous Film-Forming Foam Formulation**

*C. Ward, Q. Hu, J.C. DeWitt, East Carolina University / Pharmacology and Toxicology*

Aqueous film-forming foams (AFFFs) are mixtures of per- and polyfluoroalkyl substances (PFASs) used extensively as fire suppressants. While several PFASs, have been classified as immune hazards to humans, few studies have evaluated the effects of AFFF exposure in vertebrate models. We hypothesized that oral exposure to AFFF, like single PFASs, would suppress the T cell-dependent antibody response (TDAR) and induce peroxisome proliferation, a biomarker of exposure. Adult female and male C57BL/6 mice were given AFFF (0, 1.88, 3.75, 7.5, 10 mg/kg or 7.5 mg/kg perfluorooctanoic acid as a positive control) via gavage for 10 days. Body weights were collected daily for 15 days and urine was collected prior to the first gavage, and 48 hours, 72 hours, 5 days, 10 days, and 16 days after the first gavage. One day after dosing ended, mice were immunized with sheep red blood cells to elicit the antigen-specific IgM antibody response. Five days after dosing ended, blood/sera and organs were collected and frozen for additional analyses. Spleens were immediately prepared for enumeration of B and T cell populations by flow cytometry, liver peroxisome proliferation was determined from frozen liver samples, and TDAR was determined by ELISA. Body weights of males exposed to 7.5 and 10 mg/kg of AFFF were statistically ($P < 0.0007$) reduced by about 15%, on average, compared to the 0 mg/kg group; female body weights did not differ by dose. Relative liver weights were statistically ($P < 0.0001$) increased 50-200% in males and 37.5-193% in females relative to the 0 mg/kg group. Liver peroxisome proliferation was increased 2- to 12-fold in all dose groups relative to the 0 mg/kg group. Spleen cellularity and splenic lymphocyte subpopulations did not differ by dose. The TDAR was suppressed 18.8% and 11.1% in males and 12.6% and 16.5% in females by exposure to 7.5 and 10 mg/kg of AFFF, respectively, relative to the 0 mg/kg group. We concluded that this PFAS mixture is more potent than single compounds, i.e., PFOA alone, at increasing liver weight, inducing peroxisome proliferation, and suppressing the TDAR and males appear to be more sensitive to effects of AFFF exposure than females for most endpoints evaluated. Additional studies are warranted to determine the most appropriate methods for assessing the human health risks of exposure to AFFF.

**75 The Toxicology of 6:2 Fluorotelomer Sulfonate (C6F13CH2CH2SO3-, 6:2 FTSA)**

*R.C. Buck, S. Gannon, The Chemours Company / Fluoroproducts*

6:2 Fluorotelomer sulfonate, C6F13CH2CH2SO3-, 6:2 FTSA, has been reported in the environment. It may originate from its direct use or from degradation of precursor substances in the environment such as short-chain fluorotelomer surfactants used in fire-fighting formualtions. The acute aquatic toxicity to fish, invertebrates and algae have been determined. In a 90-day early life-stage rainbow trout study, the NOEC was 2.62 mg/l based on mean, measured concentrations and first day of hatching. The LOEC (lowest observed effect concentration) and MATC (maximum acceptable toxicant concentration) for the same endpoint were 4.85 and 3.56 mg/L, respectively. A guideline study (OECD TG 305) that included the addition of a dietary exposure conducted under GLP was conducted to evaluate the bioconcentration and bioaccumulation potential 6:2 FTSA. Exposure conditions included a dilution water control, 1 ug/L and 10 ug/L aqueous exposures and a 10 ug/kg dietary exposure with a 56 day uptake phase followed by a 28 day depuration phase. Tissue residues of the test substance in whole fish were evaluated at multiple time points during both study phases. The test results indicated that the bioconcentration and bioaccumulation potential of the test substance is low and substantially less than any existing regulatory triggers. Similarly, the acute oral, dermal and inhalation toxicity, genotoxicity and repeated dose toxicity in rats have been studied. This paper will present the results of these studies and compare them with study data for perfluorooctane sulfonate, $C_8F_{17}SO_3^-$, an eight perfluoroalkane sulfonate and perfluorohexane sulfonate, $C_6F_{13}SO_3^-$, a six perfluoroalkane sulfonate.

**76 Evaluating whether novel PFASs are safer alternatives with a new in silico tool**

*W. Cheng, University of Pittsburgh; C.A. Ng, University of Pittsburgh / Civil & Environmental Engineering*

Per- and polyfluoroalkyl substances (PFASs) are a diverse group of chemicals that have been widely used in industrial and consumer products for decades. Among them, long-chain perfluorinated alkyl acids are now recognized as highly persistent, bioaccumulative and toxic contaminants. Growing concern regarding their negative effects has led to a phasing out of the production of long-chain PFASs. To take their place, manufacturers have started using PFAS alternatives such as perfluoroether carboxylic acids (PFECAs) and perfluoroether sulfonic acids (PFESAs). However, very little information is available about the bioaccumulation potential and toxic effects of these replacement compounds. Given the vast number of PFASs, a rapid and reliable method to predict the behavior of these chemicals within organisms would be of great benefit. Our previously developed physiologically based pharmacokinetic (PBPK) model has shown that the interaction between PFASs and proteins such as serum albumin, liver-type fatty acid binding protein (LFABP), and organic anion transporters plays a critical role in determining the bioaccumulation of PFASs. In this study, we selected LFABP as our target protein and developed a workflow that combines molecular docking and molecular dynamics simulation approach to estimate the binding affinity of a total of fifteen legacy and alternative PFASs for two LFABPs (i.e., rat and human LFABP). The predicted results were compared with experimental data extracted from three different studies. There was good correlation between predicted free energies of binding and measured binding affinities, with the correlation coefficients being 0.96, 0.97, and 0.78 for three studies, respectively. This indicates that our method is able to rank the protein binding affinities of PFASs. Among the five novel PFASs (i.e., EEA, GenX, ADONA, F-53, and F-53B), our results suggest that EEA and ADONA are at least as strongly bound to rat LFABP as perfluoroheptanoic acid (PFHpA), and to human LFABP as perfluorooctanoic acid (PFOA); F-53 and F-53B both have similar or stronger binding affinities compared with perfluorooctane sulfonate (PFOS). This indicates that these alternatives could be as bioaccumulative as legacy PFAS and are not necessarily safer alternatives.

**77 Investigating gene expression in male and female zebrafish after perfluorooctane sulfonate (PFOS) exposure**

*M. Khazaee, C.A. Ng, University of Pittsburgh / Civil and Environmental Engineering*

Perfluorooctane sulfonate (PFOS) is a member of the anthropogenic class of per- and polyfluorinated alkyl substances (PFAS) that have been used in domestic and industrial products for more than 50 years. Many studies reported detectable concentrations of PFOS in water, sediments, humans, mammals, and fish around the world. The zebrafish (*Danio rerio*), is a small freshwater fish considered as an appropriate vertebrate model for investigating the toxicity of compounds. Previous investigations showed the tissue-specific bioaccumulation in male and female zebrafish, potentially due to interactions between PFAS and fatty acid transporters. PFAS strongly bind to proteins in plasma and other tissues like liver, such as apolipoproteins and fatty acid binding proteins (FABPs). Previous studies indicated that exposure to perfluorononanoic acid alters the expression of FABPs and peroxisome proliferator-activated receptors in both sexes of zebrafish. Therefore, this study was designed to investigate whether PFOS exposure affects the expression of genes associated with lipid metabolism (*fabp1a*, *fabp2*, and *fabp10*) and genes involved in the nervous system (*hdac6*, *chat*, and *ache*) in different tissues of both genders of zebrafish. Male and female zebrafish (n = 142) were randomly divided into three groups of control, low (0.1 mg/L), and high (1 mg/L) doses. Each group had three replicates per treatment, and ~ 16 fish per replicate. Fish were exposed to PFOS for 30 days and sampled on the $1^{st}$, $7^{th}$, $14^{th}$, and $30^{th}$ days of the experiment. Four fish from each replicate were dissected on each sampling day, and liver, heart, brain, muscle, intestine, and ovary were extracted and stored at −80 $^{o}$C for RNA extraction by real-time quantitative reverse transcription PCR (qPCR). Selecting a proper housekeeping gene is essential to maximize the PCR efficiency. Appropriate housekeeping genes for use as normalizers for qPCR should be stably expressed under different experimental conditions. To identify the most efficient housekeeping genes for our study, *tbp*, *bactin1*, *bactin2*, *mob4*, *eef1a1b*, and *ism12b* genes were evaluated. Based on the results, bactin1, bactin2, and tbp showed high efficiency and less variability, respectively. Moreover, results indicate differences in expression of genes associated with lipid metabolism and neural function, due to differences in both exposure concentration and gender.

**78 PFHxA Human Health Risks, Margin of Safety, and Comparison with PFOA**

*A. Luz, L. Tolbert, J. Anderson, P.E. Goodrum, Integral Consulting Inc.; D. Farrar, CCERT Ltd; S. Korzeniowski, FluoroCouncil / BeachEdge Consulting*

Perfluorohexanoate (PFHxA) is a short-chain perfluorinated carboxylic acid (PFCA) and a primary degradation product of the manufacturing intermediates used to produce some fluorinated polymers and fluorotelomer-based products today. The fluorochemistry industry specifically moved away from chemistries that relied on or resulted in long-chain PFCAs, switching to these shorter chain substances with improved hazard profiles. Because of the high stability of the carbon-fluorine bond, PFHxA is not expected to easily break down in the environment, and low concentrations of PFHxA have been detected in some environmental samples. The stability and environmental occurrence of PFHxA necessitates a clear understanding and communication of human health risk, including for the potentially most susceptible subpopulations due to higher exposures—infants and children. This presentation will summarize the available information on exposure and toxicity of PFHxA, and, relying on peer-reviewed, regulatory, human health toxicity values and human biomonitoring data, a corresponding margin of safety will be developed. Finally, methods for comparing properties of PFHxA and perfluorooctanoate (PFOA) will be presented. The available toxicological and kinetic data indicate that the relative potency of PFHxA is much lower than that of PFOA. The highest dose to humans that is unlikely to be associated with adverse effects differs between the two compounds by more than 5 orders of magnitude. Typical human exposure levels result in a drinking water threshold level for PFHxA that is almost 32,000 times higher than the USEPA lifetime health advisory for PFOA.

**79 Fluoropolymers: Polymers of Low Concern That Are Unique PFAS**

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Per- and poly-fluoroalkyl substances, PFAS, are currently the focus of researchers and regulators due to widespread presence of some PFAS (e.g., perfluorooctane sulfonate, PFOS, and perfluorooctanoic acid, PFOA) in the environment and biota, including humans. Fluoropolymers, such as polytetrafluoroethylene (PTFE), constitute a distinct class within the polymeric category of the PFAS group. Fluoropolymers are resistant to chemical, hydrolytic, oxidative, photochemical and biological degradation. They are thermally stable within their intended processing temperatures (e.g., 260°C for PTFE). Fluoropolymers have negligible residual monomer, low molecular weight oligomer, or leachable content. Fluoropolymers have high molecular weights well over 100,000 Da, are practically insoluble in water and are not mobile or subject to long-range transport in the environment. Their very high molecular weight prevents fluoropolymers from crossing the cell membrane and thus they are not bioavailable or bioaccumulative. The nontoxic nature of PTFE is supported by numerous Good Laboratory Practice (GLP) studies including acute and subchronic systemic toxicity, irritation, sensitization, local toxicity on implantation, cytotoxicity, in vitro and in vivo genotoxicity, hemolysis, complement activation, and thrombogenicity. Clinical studies of patients receiving permanently implanted PTFE-containing medical devices demonstrate no chronic toxicity or carcinogenicity, reproductive, developmental or endocrine toxicity. Fluoropolymer medical devices have been implanted in over 40 million patients for over 40 years. This poster includes fluoropolymer biocompatibility/toxicology, human clinical, and physical-chemical-thermal-biological data to show that fluoropolymers satisfy globally recognized assessment criteria to be considered as “Polymers of Low Concern” and to be recognized as being a low hazard class of PFAS. Fluoropolymers, therefore, are distinctly different from the other polymeric and non-polymeric classes of PFAS and should be separated from all other classes of PFAS for hazard assessment or regulatory actions. Grouping all classes of polymeric and non-polymeric PFAS together for restriction or regulation is not scientifically appropriate. Fluoropolymers, as polymers of low concern, are uniquely benign PFAS.

**80 Effect of sample storage on the quantitative determination of PFAS – observation of analyte interconversions during storage.**

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Sample, extract and standard stability information is critical for the accurate measurement of contaminants. In the present study, we measured the effect of sample type and storage temperature on the stability of per- and polyfluorinated alkyl substances (PFAS)in water. Spiked reagent water, surface water, and effluent samples were stored in HDPE container at +20?C, 4?C, and -20?C over a period of up to 100 days. C4-C14 perfluorinated carboxylates, C4-C10 and C12 perfluorinated sulfonates, 4:2, 6:2 and 8:2 fluorotelomer sulfonates, three perfluorooctane sulfonamides (PFOSA, N-MeFOSA, N-EtFOSA), two perfluorooctane sulfonamide ethanols (N-MeFOSE and N-EtFOSE), and two perfluorooctane sulfonamideacetic acids ((N-MeFOSAA and EtFOSAA) were included in the study. Overall, perfluorinated carboxylates, sulfonates, and fluorotelomer sulfonates did not display significant concentration changes in reagent water, surface water and effluent samples over the study period and at all temperature conditions. There was a steady increase in the concentrations of NMeFOSAA and NEtFOSAA in both surface water and effluent samples over the study period at +20?C, and 4?C. The behavior was not observed in reagent water samples and indicated the impact of sample matrix on the stability of polyfluorinated compounds during storage. In addition, the increases in concentrations of NMeFOSAA and NEtFOSAA tracked the decreases in the concentrations of N-MeFOSE and N-MeFOSA, N-EtFOSE and N-EtFOSA respectively indicating potential for analyte conversion during sample storage. This is the first time such analyte conversion is reported in samples under storage and demonstrates the importance of assessing stability of polyfluorinated compounds in environmentally relevant matrices.

## Bayesian Network Applications for Environmental Risk Assessment and Management

**81 An Introduction to Bayesian Networks for Environmental Risk Assessment and Management**

*W.G. Landis, Western Washington University / Institute of Environmental Toxicology; J.F. Carriger, Jr., USEPA / National Risk Management Research Laboratory*

Bayesian networks (BNs, also known as Bayesian belief networks) are a means of describing cause and effect using directed acyclic graphs where the interactions between the nodes are described using conditional probability tables. That is a way of saying that that there are nodes (steps in a cause-effect model), which are connected by lines of influence, and the interactions are described using probability tables that take into account all possible combinations of inputs to generate the probability distributions for each possible outcome. Bayesian networks inherently incorporate uncertainty and can use wide varieties of data, from precise determinations of molecular interactions to expert elicitation. B. Marcot was an early pioneer in the use of BNs for environmental management for the US Forest Service. The use of BNs in ecological risk assessment began in the early 2000s pioneered by B. Hart and C. Pollino in Australia. Since 2010 a number of papers have been published on the use of BNs to evaluate management options, to evaluate risk, to provide a more quantitative framework for the relative risk model, and to become a part of adaptive management. This session includes presentations by developers and users of Bayesian networks to estimate risk, calculate the outcomes of management alternatives and the use of such techniques to make policy decisions. Likely topics include estimating the risk due to contaminants, invasive species, oil spills, climate change, the inclusion of adverse outcome pathways as part of ecological risk assessment, and the evaluation of management alternatives. The use of BNs as part of developing long-term policy in collaboration with managers and stakeholders will be presented. The big questions are now where do we go from here and the adoption of BNs to advance ecological risk assessment and management.

**82 Data-Informed City-Level Adaptive Management: A Bayesian Approach for Quantifying Climate Change Risk and Resilience in Charleston, SC**

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The greater Charleston Harbor region is highly susceptible to the projected impacts of climate change due to low lying geography, a disparate socioeconomic spectrum, and invaluable ecosystem services. In an effort to become climate change resilient, the City of Charleston, SC is conducting an "all hazards" vulnerability assessment to geospatially identify populations and assets (e.g. economic, historic, and ecosystem services) vulnerable to sea level rise, precipitation extremes, extreme heat, and storm surges. The presented research highlights the use of the Bayesian Network-Relative Risk Model as a means to quantify climate change risk and resilience beyond a semi-quantitative vulnerability assessment. The BN-RRM is parameterized using inherent community knowledge gathered via facilitated workshops and the Multi-level Parameterization Risk Assessment Framework. The parameterization framework breaks down a primary stressor (e.g. climate change) into its secondary (e.g. sea level rise, extreme precipitation) and tertiary (e.g. nuisance flooding, habitat alteration) stressors. Tertiary climate change stressors are consequences of the secondary stressors, just as the secondary stressors are regional consequences of global climate change. As an adaptation of the BN-RRM, the presented multi-stressor model incorporates resilience parameters as control factors that modulate how risk is carried through the model. These control factors serve as "filters" controlling the degree of exposure to stressors and intensity of effects. Control factors represent resilience characteristics (i.e. plan/prepare, absorb, recover, and adapt), management practices, and/or personal actions that, when properly implemented, reduce exposure to a stressor and/or the impact of an effect, which effectively reduces risk to the assessed endpoint. If managers (e.g. city, emergency, or environmental managers) are to manage within a complex system, their decisions should be informed by assessments that, at the very least, attempt to evaluate the system as a whole. While single stressor risk assessments are inherently easier to conduct, management decisions based on one or a suite of single stressor risk assessments may not be effective due neglected sources of compounding risk. The BN-RRM approach provides the City of Charleston with a decision support tool to systematically quantify the multi-stressor risk-resilience relationship, an otherwise emergent property of a complex system.

**83 Quantification of AOPs by Bayesian network modelling: Linking chemical exposure to adverse outcomes in Lemna minor populations**

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Adverse Outcome Pathways (AOPs) has gained international recognition as a systematic approach for capturing existing toxicological knowledge to transparently link mechanistic data to toxicity endpoints. Nevertheless, qualitative AOPs are not directly suitable for quantitative risk assessment. Quantitative AOPs (qAOP) should define the relationships underlying transition from one KE to the next sufficiently well to allow quantitative prediction of the probability or severity of the AO occurring for a given activation of the MIE. We have developed a Bayesian Network (BN) model to quantify a recently proposed AOP, which links the mode of action of the model respiratory and photosynthesis uncoupler 3,5-dichlorophenol (DCP) to adverse outcomes in the aquatic plant *Lemna minor.* The BN model is based on data from a laboratory experiment exposing *L. minor* to DCP in 8 concentrations with 3 replicates. The measured response variables include OXPHOS (oxidative phosphorylation), ROS (reactive oxygen species), ETR (electron transfer rate), Fv/Fm (maximum quantum yield of photosystem II), LPO (lipid peroxidation) and number of fronds (leaves). The proposed AOP is a network consisting of three chains with the same chemical stressor (DCP) and AO (fronds number). All AOP components are defined in the BN as nodes with discrete states. Each node is quantified by a probability distribution across these states, and the causal links (Key Event Relationships) are quantified as conditional probability tables (CPTs). The probability distributions of the CPTs are obtained from dose-response regression models fitted to the experimental data and their estimated uncertainty. The BN was tested by examining the response of the nodes to different concentrations of DCP and observing the exposure-response relationships. All KEs and AOs of the BN responded to increasing the MIE (DCP concentration) in accordance with the conceptual model. Increasing DCP resulted in monotonous decrease in fronds number in all three pathways. Sensitivity analysis indicated that the pathway OXPHOS → ETR → fronds number was the most sensitive to changes in DCP concentration, while the pathway ROS → LPO → Fronds no. was the least sensitive to the DCP concentration. Planned further developments of this BN model include linking the AO to population-level endpoints with regulatory relevance, and linking the chemical stressor to an Aggregate Exposure Pathway.

**84 Integrating climate change stressors and human health and well-being endpoints into a Bayesian network relative risk model**

*E. Lawerence, A.J. Markiewicz, W.G. Landis, Western Washington University / Institute of Environmental Toxicology*

Climate change is expected to have widespread impacts on ecosystem services in the Salish Sea. In this research, we focused on the question of how stressors generated by climate change affect contaminant toxicity to species in the Skagit River Watershed. Specifically we assessed how those combined effects potentially influence risks to the river's ecosystem services that, in turn, impact human health and well-being. To answer this question, we are conducted an ecological risk assessment using the Bayesian network relative risk model (BN-RRM). It is a quantitative, probability-based model that calculates complex relationships between ecological variables to provide estimates of risk to valued receptors (endpoints). The Skagit River Watershed contains important habitats for native salmon species and bald eagles (*Haliaeetus leucocephalus*). These species provide numerous ecological, economic, cultural, and spiritual services to humans. Its floodplains also provide fertile, highly productive croplands, making it an important agricultural center in the region. Pesticide use on croplands in the watershed pose potential threats to these non-target species that may increase in severity with climate change. Increasing water temperature, decreasing dissolved oxygen levels, and changes in seawater pH are of particular concern, as are changing river and stream flows, increasing storm event frequency and intensity, and sea level rise. These stressors have potential to impact human health and well-being endpoints such as human well-being, water quality, salmon fisheries, tribal cultural and community health indicators, recreation areas, tourism, agriculture, boating, fishing, and shellfish harvesting. The BN-RRM enables us to calculate the risks posed by various stressors on these select endpoints in the Skagit River watershed due to climate change. The BN-RRM can also serve as a useful tool for resource managers and decision-makers as part of an adaptive management process and to direct future research efforts in the watershed, as well as in other watersheds in the Salish Sea region.

**85 A regional scale ecological risk framework for environmental flow evaluations.**

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Environmental Flow (E-flow) frameworks advocate holistic, regional scale, probabilistic E-flow assessments that consider flow and non-flow drivers of change in a socio-ecological context as best practice. Regional Scale ecological risk assessments of multiple stressors to social and ecological endpoints, that address ecosystem dynamism, have been undertaken internationally at different spatial scales using the relative-risk model since the mid 1990's. With the recent incorporation of Bayesian belief networks into the relative-risk model, a robust regional scale ecological risk assessment approach is available that can contribute to achieving the best practice recommendations of E-flow frameworks. PROBFLO is a holistic E-flow assessment method that incorporates the relative-risk model and Bayesian belief networks (BN-RRM) into a transparent probabilistic modelling tool that addresses uncertainty explicitly. PROBFLO has been developed to evaluate the socio-ecological consequences of historical, current and future water resource use scenarios and generate E-flow requirements on regional scales spatial scales. The approach has been implemented in two regional scale case studies in Africa where its flexibility and functionality has been demonstrated. In both case studies the evidence based outcomes facilitated informed environmental management decision making, with trade-off considerations in the context of social and ecological aspirations. This paper presents the PROBFLO approach as applied to the Senqu River catchment in Lesotho and further developments and application in the Mara River catchment in Kenya and Tanzania. The ten BN-RRM procedural steps incorporated in PROBFLO are demonstrated with examples from both case studies. PROBFLO can contribute to the adaptive management of water resources, and contribute to the allocation of resources for sustainable use of resources and address protection requirements.

**86 Multiple Stressor Ecological Risk Assessment of a Highly Regulated Water Catchment in the Context of South African Legislation**

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The uMngeni River, is a highly regulated, socio-ecologically important catchment in KwaZulu-Natal, South Africa, where more than four million people live and depend on the system. Increasing urban growth and economic investment in an area of strategic national importance, has resulted in increasing demands for water supply to sustain social and economic development. Land use in this catchment includes rural communities, high intensive agriculture, forest plantations, industries and urban centres. National legislation requires the achievement of a balance between water provision for all water users and protection of water resources. The Bayesian Network Relative Risk Model (BN-RRM) is used to model multiple stressors in this dynamic catchment to four endpoints: two ecological endpoints (Maintenance of river and riparian connectivity, Control of alien invasive plant species), and two social endpoints (Maintain Basic Human Needs and Maintain ecosystem for recreation and tourism).
The study area was delineated into six risk regions linked to location of impoundments. The endpoints are measured against the legislated water resource quality objectives (RQOs) for the river resources, to determine if the RQOs are achievable under the current scenario and alternative scenarios. This case study integrates local hydrology data, water quality data, impoundment information and data, water use statistics and land use information, to generate risk profiles for the selected endpoints. This is a probabilistic framework, that adopts a holistic approach to evaluate trade-offs between selected ecological and social endpoints within the context of legislation. Results under the current situation indicate that there are differences in risks to the selected socio-ecological endpoints in each risk region, which are influenced by varying environmental conditions in each risk region. Land use and drought conditions are significant considerations for habitat quality and habitat availability. Future scenarios such as increased urban growth and climate change, indicate regional variations associated with a synergy of various stressors. Risk profiles for the aforementioned future scenarios suggest that achieving the legislated RQOs may be challenging, unless there are specific interventions.

**87 Combining Bayesian networks and conceptual models for Superfund remediation support**

*J.F. Carriger, Jr., USEPA / National Risk Management Research Laboratory; R.A. Parker, US Environmental Protection Agency / National Risk Management Research Laboratory*

Conceptual models play a central role in Superfund remediation. They are used as a communication tool and a platform for quantitative models. Bayesian networks offer many opportunities for supporting conceptual and quantitative modeling in Superfund remediation. However, their application to Superfund risk assessments and management is still rare. A framework will be presented that combines Bayesian network knowledge engineering and development with requirements and recommendations for Superfund conceptual models. We call the resulting products conceptual Bayesian networks (CBNs). A development process for Superfund CBNs will be introduced that can support all phases of site investigation and cleanup. Learning is incorporated into the process for updating knowledge of the model structural components and connections as new data and understanding becomes available. Inferences to support decision making with a CBN will be presented including causal pathway analysis from sources of stressors to receptors of concern and capabilities for examining the causal influence of remediation interventions in breaking exposure pathways. The CBN process is designed for adaptability to multiple sites and stressor-types as well as comprehensiveness for adequately incorporating required legal and scientific guidance for site assessments. Components and connections that are broadly transferrable will be shown. The focus of the presentation is on the qualitative side of Bayesian network development and inferences, which is necessary for subsequently developing quantitative models. The potential value added of a CBN for quantitative risk assessments will be demonstrated. The flexibility of CBNs allows adaptation across all phases of Superfund assessments and can support management decision making, uncertainty evaluation, and communication from the structural and quantitative capabilities of Bayesian networks.

**88 Relative Risk Models Using Bayesian Networks: Application, Communication and Regulatory Use**

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Historical mercury release occurred on the South River, Virginia, between 1929 and 1950 from a former DuPont facility. Legacy mercury in the potentially impacted areas has been the subject of numerous studies over the last four decades. The South River Science Team (SRST), a multi-stakeholder group, was established in 2001 to investigate the potential impacts to the aquatic and riparian terrestrial systems along the South River and a portion of the South Fork Shenandoah River. A model framework was developed with input from the SRST based on Bayesian Network-Relative Risk Models (BN-RRMs). The BN-RRMs for two ecological receptors (i.e., Smallmouth Bass and Carolina Wren) and ecosystem services (i.e., Swimming, Boating, and Fishing) were applied to support risk and remedial decisions for the South River. These BN-RRMs integrated contributory risk factors and quantified overall risk scores for five contiguous risk regions, including an upstream reference region located approximately 10 miles upstream from the former DuPont facility, as well as portions of the South River and the South Fork Shenandoah River extending 30 miles downstream of the former DuPont facility. The risk regions were configured to be as consistent as possible with natural breaks based on land use and hydrogeology, the upstream-to-downstream remedial approach and existing short- and long-term monitoring stations. The BN-RRMs were applied primarily to evaluate: 1) the relative importance of contributory factors besides mercury and 2) "what if" scenarios regarding anticipated system response to the remedial approach/extent. The use of the select BN-RRMs was realized as intended, simply as a tool to generate one of several lines of evidence. The modelling results showed that factors other than mercury contributed to overall risks, relative risks varied among the risk regions, and planned remedial measures would achieve varying degrees of risk mitigation among the risk regions. As such, the use of BN-RRMs facilitated communication of a holistic approach to risk evaluation and provided a predictive capability to support adaptive management approaches to remedial decisions. In this presentation, the following aspects of the models and their applications will be shared: 1) modeling framework, 2) public and regulatory receptiveness; 3) transparency, limitation, and communication; and 4) overall utility in a regulatory context.

# Per- and Polyfluoroalkyl Substances: Recent Advances and Future Directions – Part 2

### 89 Measurement, occurrence and applications of GenX (HFPO-DA), a replacement for PFOA

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Per-and polyfluorinated alkyl substances (PFAS) are a focus of study due to widespread use, prevalence in the environment, and bioaccumulation and health effects of longer chain perfluoroalkyl acids (PFAAs) such as PFOA and PFOS. Phaseout of PFOS and PFOA, and their replacement by newer chemistry means that these "novel" PFAS are being detected in the environment. For example, PFOA in fluoropolymer manufacture is being replaced with a diverse set of per-/polyfluoroether carboxylic acids including GenX (2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy) propanoic acid or HFPO-DA) , ADONA (3H-perfluoro-3-[(3-methoxy-propoxy)propanoic acid]), PMPA (tetrafluoro-2-( hexafluoro-2-(trifluoromethoxy)propoxy]-propanoate) and others. Chrome plating alternatives for PFOS include F-53B (6:2 chlorinated polyfluorinated ether sulfonate) and others. More data is needed on occurrence, fate, transport and health effects of these replacment PFAS in order to inform risk asessment. Of these, GenX is under increased scrutiny, especially in North America after the discovery of HFPO-DA in watersheds adjacent to a fluoropolymer manufacturing facility in North Carolina[1]. There is a great need for increased monitoring of HFPO-DA in the environment, occurrence and bioaccumulation in fish/wildlife, and fate in sewage treatment. The use of oxidative assays such as the total oxidizable precursor (TOP) assay can also shed light on ultimate environmental fate. This study reviews GenX use, analytical methods, occurrence in the ecosystem and in wastewater treatment plants, and results from TOP analysis. Methods for HFPO-DA were developed and validated in water and soil using isotope dilution LC-MS/MS. 250mL aqueous samples were fortified with $^{13}C_3$-HFPO-DA, extracted using weak anion exchange (WAX), and concentrated prior to analysis. 5g soil samples were fortified with $^{13}C_3$-HFPO-DA, extracted using methanol, cleaned up using WAX, and concentrated. Extracts were analysed using a Waters Acquity UPLC interfaced with a Waters TQ-S tandem mass spectrometer. Validation in aqueous samples resulted in a recovery of 97% with an RSD of 5% and method detection limits < 5 ng/L. Solid samples showed a recovery of 111% with an RSD of 15%, and method detection limits < 0.5 ng/g. Data on occurrence in the ecosystem and wastewater treatment plants, and fate of HFPO-DA in the TOP assay will be presented. References Strynar, M., Dagnino, S. et al. (2015). Environ. Sci. Technol. 49, 11622–11630.

### 90 Aerobic Biodegradation of Short-Chain Fluorotelomer Based Methacrylate Polymers Is Not A Significant Source Of Environmental Short-Chain PFCAs

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Short-chain fluorotelomer based methacrylate polymers are a class of side-chain fluorinated polymers used for a variety of commercial applications, including some food contact packaging materials and treatment of carpet and rug fibers. It has been suggested that the degradation of these polymers could be a source of short-chain perfluoroalkyl carboxylates (PFCAs) to the environment and there is increasing concern regarding the stability of these polymers in the environment. Per USEPA requirements under the Toxic Substances Control Act, the aerobic biodegradation of a specific fluorotelomer-based methacrylate polymer, identified as "Nuva RP2116 GA", was evaluated following USEPA OPPTS 835.4100 (2008) and OECD 307 Aerobic and AnaerobicTransformation in Soil (2002) guidelines over ~15 months in four natural soil conditions. Potential degradation products were selected for measurement under direction from USEPA and included fluorotelomer alcohols 5-2s FTOH and 6:2 FTOH (by GCMS/MS analysis) and perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid, C6-2 fluorotelomer carboxylic acid, C6-2 fluorotelomer unsaturated carboxylic acid, and C5-3 fluorotelomer carboxylic acid (by LC-MS/MS analysis).6-2 FTOH, which is considered to be the primary initial metabolite associated with the biological transformation as well as a potential low level impurity, was rapidly transformed in all four soils and remained at a low levels in the test material. Kinetic parameter estimates include conditions in which a plausible fraction of 6-2 FTOH equivalents may have been present as an impurity in the test substance. The dominant intermediate metabolite, 5-2s FTOH, was observed mainly during the first 3 to 6 months. The dominant terminal metabolites were PFPeA and PFHxA. At the conclusion of the test the sum of all potential degradation products was less than 0.04% of 6-2 FTOH equivalents in all four soils. A first order kinetic model applied to residual 6-2 FTOH equivalents measured in the initial period of degradation (prior to reaching a plateau) supports estimated half-lives of thousands of years in soil. Transformation rates would be even slower in soils with less aerobic biological activity and studies under anaerobic conditions are underway. Aerobic transformation in soil of short-chain fluorotelomer based methacrylate polymers are, therefore, not likely a significant source of short-chain PFCAs currently detected in the environment.

### 91 The sorption of PFOA, PFOS and PFHxS in soils

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Emerging contaminants, such as perfluorinated alkyl substances (PFASs) are of increasing concern in the environment due to their stability, longevity, bioaccumulation and potential toxicity. These attributes have led perfluorooctanesulphonic acid (PFOS) to be placed on the Stockholm Convention for persistent organic pollutants (POPs) and perfluorooctanoic acid (PFOA) and perlfuorohexane sulphonate (PFHxS) are currently being considered. Concentrations of PFASs in soils vary greatly – from pg/kg to mg/kg – but are particularly high in contaminated sites such as fire-fighting training grounds and airfields (mg/kg). Once exposed in the environment their behaviour and interactions with soil are not completely understood. Small scale studies, generally comprising < 10 soils, in terrestrial systems have indicated that there are relationships between retention of PFASs and soil organic matter content, clay content and iron oxides. In this study PFOS, PFOA and PFHxS were tested for their sorption capacity to 170 characteristically different soils from an Australian national soil archive. The soils were randomly selected to provide a wide range of properties such as pH, total carbon content, cation exchange capacity and clay content. The aims of this study were to: establish soil-water partitioning coefficients ($K_d$) for a wide range of soils; establish which soil characteristics control the binding of these PFASs; and develop simple rapid methods to predict PFAS partitioning in soils. The use of simple linear regression, multiple linear regression (MLR) and partial least squares regression in conjunction with soil spectra from diffuse reflectance mid-infrared Fourier transform spectroscopy (MIR-PLSR) was used to model the sorption of PFOS, PFOA and PFHxS in the soils. Soil organic carbon content was not adequate to explain partitioning of these chemicals in soils, indicating that normalising partition coefficients to organic carbon content only, is less effective than considering a wider range of soil properties. The MIR-PLSR modelling technique was found to be an extremely rapid, cheap and robust method to predict partitioning of these compounds across a wide range of soils.

**92 Novel Fluorinated Polymer Surfactants in Biosolids From Wastewater Treatment Plants Across Canada and Source Linkages to Biosolid-Augmented Soils**

*R.J. Letcher, Environment and Climate Change Canada / Ecotoxicology and Wildlife Health Division; S. Chu, S. Smyth, Environment and Climate Change Canada*

Side-chain fluorinated polymer surfactants are per-/poly-fluoroalkyl substances (PFASs) and are the main components of fabric protector sprays and used on furniture and textiles. These fluorinated polymers may be a source, as a result of their degradation and subsequent formation, of perfluorinated carboxylic acids (PFCAs) and perfluorinated alkane-sulfonates (PFSAs). We have shown that available fluorinated surfactant products, pre- and post-2002 Scotchgard fabric protector (3M Company), can be metabolically degraded in an in vitro assay based on Wistar-Han rats liver microsomes, to metabolites containing the N-methyl-perfluorobutanesulfonyl (S1) and N-methyl-perfluoroctanesulfonyl (S2) chemical moieties, respectively, which are precursors to perfluoro-1-octane sulfonamide (FOSA) and perfluoro-1-butane sulfonamide (FBSA), respectively. Studies have shown the presence of various PFASs including S1 and S2 in agricultural soils in southern Ontario, Canada, where wastewater treatment plant (WWTP) sourced biosolid application had occurred and not occurred. The main side-chain fluorinated polymer components in pre-Scotchgard (S1) and post-Scotchgard (S2) were shown to be source linked to the biosolid application. S1 was detected in 100% of the soil samples from biosolid-augmented agricultural sites at a mean concentration of 236 ng/g d.w., and at concentrations much greater than in soil samples from the two sites where no biosolid had been applied (mean concentrations of 3.02 and 17.36 ng/g d.w.). The concentrations of S1 and S2 in soil samples were also much greater than the total concentration of other PFASs (including PFOS) that were measured. We further investigated S1 and S2 side-chain fluorinated polymer contaminants, and FOSA, FBSA and other PFASs (i.e. 13 PFCAs, 5 PFSAs and N-MeFOSA and N-EtFOSA) in biosolid samples collected in 2014 to 2017 from 20 WWTPs serving urban centres across Canada. S1 and S2 were found in the biosolid samples. PFAS concentrations in biosolid samples were also a function of treatment type, including digestion (aerobic or mesophilic anaerobic), dewatering, alkaline stabilization and/or pelletization. These findings raise a concern regarding the use of biosolids to enrich agricultural soils, which appears to be a major source vector for Ontario soil contamination with these new PFAS contaminants, and may be a human and/or terrestrial wildlife exposure issue crops grown in these biosolid-augmented fields.

**93 Assessing spatial trends and depth profiles of PFAS-contaminated soils from an AFFF-impacted site**

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Soil cores from a former fire training area were analyzed for a wide variety of poly- and perfluoroalkyl substances (PFASs) using liquid chromatography-quadrupole time of flight high resolution mass spectrometry (LC-QTOF-HRMS). A novel extraction method was used to enhance extraction of cationic and zwitterionic PFAS from soils, as previous methodology was insufficient for complete extraction of strongly sorbing species. The novel extraction procedure consisted of two rounds of 0.1 M ammonium hydroxide in methanol, followed by two rounds of 0.5 M hydrochloric acid in methanol. Extracts were analyzed by LC-QTOF-HRMS, with targeted quantification of 40 PFAS compounds, and spectral library and accurate mass screening against a custom-built PFAS mass spectral library and exact mass list. Identified compounds lacking analytical standards were semi-quantified and assigned different levels of confidence based on their exact mass, isotope ratios, and fragmentation. Further, the data were screened by a nontarget R script to identify any additional PFASs. Spatial concentration trends and depth profiles were examined for detected compounds. Better extraction of all PFASs more accurately captures the total environmental concentrations and improves understanding of the variable transport properties of diverse compound classes.

**94 PFAS detection at AFFF impacted sites using a fiber passive sampler**

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Since 1950s, poly- and perfluoroalkyl substances (PFAS) have been produced for many industrial and consumer applications including aqueous film-forming foams (AFFF). Their unique chemical properties and exceptional resistance to physical-chemical and biological degradation in combination with their high production volumes led to their ubiquitous presence in the environment. In past decades, their frequent releases to the environment have caused substantial background exposure to PFASs that affect all people in U.S. As indicated by recently published data, 6 million people in the U.S. may have been exposed via drinking water to PFAS levels exceeding the water health advisories levels. Presumably, the widespread contamination of drinking water is associated with AFFF application and chemical releases from more than 600 PFAS contaminated sites around the U.S. To prevent the future PFAS exposure, an essential step, followed by subsequent corrective action (e.g. remedial projects), is to establish a quick, reliable and robust monitoring tool for the detection of PFAS at potentially contaminated sites. The use of passive samplers, which has become a routine application for hydrophobic organic contaminants is convenient for the purpose of determining dissolved PFAS concentrations in porewater. Moreover, the passive samplers provide time-weighted average concentrations in water and porewater, which can be used to assess bioavailability of PFASs at contaminated sites. Solid-phase microextraction (SPME) fibers have been successfully used previously to quantify freely dissolved concentrations of nonionic, anionic, and cationic surfactants. In this work, polyacrylate (PA) fibers were used to determine the equilibrium partitioning constant, $K_{PA\text{-}W}$ (the ratio of PFAS concentrations in solution and on the fiber at equilibrium). SPME fibers (with various PA coating) were exposed to dissolved PFASs in aqueous solution and the distribution coefficient was calculated. The log $K_{PA\_W}$ increases with increasing molar weight/ length of the carbon chain of carboxylic acids and sulfonates. Moreover, the log $K_{PA\text{-}W}$ for perfluoroamine with eight carbons in a chain was calculated and indicating strong affinity for the SPME fibers (two to three times higher in comparison with sulfonate and acid of the same carbon chain length). The calculated log $K_{PA\_W}$ for the polyfluoroosulfonates (FTS) showing a lower affinity to the SPME fiber in comparison with perfluorinated analogs.

**95 Legacy and emerging poly- and perfluoroalkyl substances (PFASs) in seabirds from Atlantic offshore and coastal environments**

*A. Robuck, Graduate School of Oceanography / University of Rhode Island / Chemical Oceanography; M.G. Cantwell, US Environmental Protection Agency; C. Gardiner, R. Lohmann, Graduate School of Oceanography / University of Rhode Island*

Poly- and perfluoroalkyl substances (PFASs) have been produced since the 1940s for use in commercial and industrial applications, including water repellant coatings, surfactants, and vinyl polymerization. PFASs demonstrate remarkable environmental persistence and bioaccumulative capacity, and have been found globally in drinking water, surface water, and biota, including birds from diverse habitats. Seabirds are particularly useful as indicators of marine ecosystem health and contamination, as their upper trophic level position allows them to assimilate resources and related biological, physical, and chemical conditions across multiple ecosystems and temporal scales. Here, PFASs were measured in juvenile birds from the Atlantic seaboard. Bird liver samples were obtained from deceased or bycatch juvenile or sub-adult herring gulls, great shearwaters, terns, and pelicans from Narragansett Bay in Rhode

Island, Massachusetts Bay off the coast of Massachusetts, and the Cape Fear River Estuary in southeastern North Carolina. Liver samples from each bird were freeze-dried and extracted using sonication, centrifugation and freezing, and purified using reversed phase solid phase extraction. Extracts were analyzed for 25 legacy and emerging ionic and neutral PFASs using liquid chromatography/tandem quadrupole mass spectrometry in electrospray ionization (ESI-) mode. Coastal birds generally contained higher concentrations of sum PFASs compared to offshore birds, with the coastal group evidencing mean concentrations of sum PFASs of 999.8 ±627 ng/g dry weight, while offshore birds displayed sum PFASs of 598.7 ±437.4 ng/g dry weight liver. PFCAs dominated the offshore group, while coastal birds contained a higher proportion of PFSAs. Concentration differences and variability in compound dominance between groups could not be attributed to trophic level as approximated by nitrogen stable isotopes, suggesting dissimilar sources may variably contribute to PFASs fingerprints observed between groups. Observed concentrations suggest abundant PFASs in seabirds from both developed coastal and remote pelagic Atlantic systems, contributed by as-of-yet uncertain pathways.

**96 Per and Polyfluoroalkyl Substances Under CERCLA**

*L. Libelo, USEPA / OLEM OSRTI SPB; S.D. Foster, K. Raffaele, USEPA / OLEM; L. Gaines, USEPA / OLEM OSRTI SPB*

Because of their long history and widespread use, per- and polyfluoroalkyl substances (PFAS) are ubiquitous contaminants in environmental media. Historical waste disposal practices, environmental release during use, and spills have led to PFAS concentrations in sites that may fall under Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), commonly known as "Superfund", authority. While PFAS are not currently listed as hazardous substances under CERCLA, analysis of soil, water, and other media at current and potential Superfund sites is increasingly identifying PFAS as potential contaminants of concern. PFAS have been detected at more than 100 sites on the National Priorities List. Many, perhaps hundreds, of additional sites may be contaminated with these chemicals, and may be eligible for remediation under CERCLA authority. Current tools for dealing with fluorochemicals at "Superfund" sites are limited. EPA is taking a number of actions to help address this situation, including: setting groundwater screening and clean-up goals for PFOA and PFOS; evaluating designation of some PFAS as hazardous substances to clarify listing and cleanup decisions and facilitate cost recovery; developing and validating analytical methods and sampling methods for quantification in different environmental media; collecting information on the number, locations and characteristics of PFAS contaminated sites; and evaluating cleanup technologies and approaches. This presentation will give an overview of EPA's activities related to PFAS under Superfund including current and ongoing efforts at characterization, potential regulation, and cleanup. The views expressed are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

## Approaches and Challenges in Sediment Toxicity Testing for Environmental Risk Assessment

**97 Evaluating risk of plant protection products to benthic invertebrates: Is there harmonization of protection goals in lieu of harmonized guidance?**

*M.J. Bradley, Smithers Viscient / Ecotoxicology*

Globally, there is a general understanding that certain chemicals in the environment pose a greater threat to benthic dwelling organisms than others, generally related to inherent physical properties of the chemical. Beyond this, however, there is little agreement in the regulations as to how risk should be assessed. From determining what chemicals pose a risk, to what species should be evaluated and how these exposures should be evaluated, various regulations all take their own course. These differences are all very well outlined in the European Food Safety Authority (EFSA) 2015 Scientific Opinion on the Effect Assessment for Pesticides on Sediment Organisms in Edge-of-Field Surface Water. But what is the end result? When considering testing requirements and applied safety factors, is there a continental difference in the ultimate protection of sediment dwelling organisms? In Europe, a single long-term chironomid exposure may satisfy testing requirements, while multiple species and exposure types may be required in the United States, all with the same goal of establishing a threshold concentration protective of invertebrate benthic populations. Variable species sensitivity as well as exposure system dynamics will undoubtedly effect individual outcomes, but there is enough commonality among these designs that a similar level of protection may also be the likely outcome. Further, any differences may become moot once relative assessment factors are applied to establish risk. This presentation seeks to evaluate the relative sensitivity of standard European and USEPA test species and their respective test designs for a number of pesticide groups. These data will further be evaluated in the context of their regulatory applications and relevant safety factors to assess if largely different levels of protection exist depending on the governing body.

**98 Spatio-temporal exposure of Plant Protection Products in OECD 219 sediment test systems – Comparison of model results with measurements**

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Sediment toxicity testing is currently revised under the premise to improve quality and consistency of regulatory environmental risk assessments. In 2015, the European Food Safety Authority (EFSA) published a scientific opinion on environmental risk assessment for sediment organisms where a water-spiked test system (OECD 219) is considered to study chronic effects on sediment organisms. Prominent test organisms are Chironomids, aquatic insects which live in and on soft sediments. Due to the design of this study, initially large gradients between the exposure in the overlying water and in the sediment layer are established. As a consequence, substantial temporal and spatial dynamics of local concentrations have to be expected, especially in the vicinity of the interface beween water and sediment where the Chironomids inhabit. To describe local concentrations in such water-sediment test systems, we simulated the transport and the redistribution of two moderately mobile ($K_{OC}$ 200 to 300 L/kg) plant protection products with the mechanistic model TOXSWA. The results of the simulation are compared with measured sediment concentrations in three depths (see poster submitted by McCoole et al. in this session). The compound properties were parameterised using values derived independently in standard tests ($K_{OC}$, $DT50_{water/sediement}$) or from literature (diffusion coefficients). Other parameters were derived from OECD 219 experimental design information. The simulations matched the measured concentrations spatially and temporally well. The simulated concentration depth profiles averaged for the layers which were measured lay almost always within the range of single measurements. Also the concentrations in the overlying water were reproduced well. The main findings are that the concentrations in the sediment show a pronounced temporal pattern and that the concentrations in the sediment are strongly depth-dependent. The dominant transport process in the sediment is obviously diffusion which, however, did not lead to homogeneous penetration of the sediment. Presuming that Chironomids live in the upper three millimetres of the sediment, they are exposed to approximately four times higher test compound concentration (total and liquid) than the average concentration in the sediment. This has important consequences for the derivation of effect endpoints of Chironomids from OECD 219 study data, which should consider the local exposure of the organisms.

**99 Influences of Environmental and Anthropegenic Contaminants on Bacterial Communities in Sediments of the West Coast of Korea**

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Bacterial communities in coastal sediments are recognized to play a significant role in the ocean ecosystem functioning and regulate relevant biogeochemical processes. Chemical contaminants, such as heavy metals, polycyclic aromatic hydrocarbons (PAHs), and alkylphenols (APs) can accumulate in sediments and (in)directly influence corresponding bacterial communities. Nevertheless, relatively few studies have investigated the link of bacterial community diversity to anthropogenic stresses, particularly in long-term aspect. In the present study, we aimed to 1) investigate spatiotemporal changes of the bacterial communities in sediments collected from the west coast of Korea, particularly including the severely polluted coastal sites along the coast, from 2010 to 2014, 2) assess the relationship between sediment bacterial communities (viz., bacterial diversity and community composition) and sedimentary pollutants, such as PAHs, APs, and heavy metals, and 3) identify the potential toxicities of AhR- and ER-active chemicals present in sediment samples. The diversity of bacterial communities was not significantly correlated with salinity, AhR-, ER-mediated activities, and concentrations of APs, whilst heavy metals and PAHs seemed to strongly influence on the structure of bacterial communities. In specific, the community of phylum Planctomycetes showed significantly negative correlations to concentrations of heavy metals and PAHs. Overall, the results suggested that the sediment bacterial community could be one potential proxy component to address the pollution status in the integrated sediment assessment.

**100 Application of different extraction methods in screening for the effects of a tropical freshwater dam sediment on the steroidogenesis of H295R cells**

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In the present study H295R human cells were used to investigate the endocrine disruptor potential of three different sediment extracts taken from a Nigerian freshwater dam (Awba Dam). Each extract (polar compounds extract, non-polar compounds extract and elutriate) permits to selectively take into consideration different group of contaminants in relation to their binding affinity with sediments particles (mainly driven by polarity), and by consequence assessing the relevance of different exposure routes that each contaminant is more likely to undergo (resuspension, direct ingestion and trophic web transfer or diffusion at the water sediment interface). After exposure to different concentration of each extract, H295R cells were evaluated with regards to expression profiles for 10 genes heavily involved in steroidogenesis (*cyp11a, cyp11b2, cyp17, cyp19, cyp21, 3β-hsd2, 17β-hsd1, 17β-hsd4, star, hmgr*) and for the synthesis of estradiol and testosterone. Our results showed similar increased expression, going from the lower to the highest concentration for *17βhsd1, 3βhsd2* and *cyp21* in the cells treated with the polar and non-polar extracts. *star*, *hmgr*, *cyp11b2* and *17β-hsd4* were slightly down regulated in the polar treatment and up regulated in the non-polar extracts treatment especially in the highest concentration. The *cyp11a* and *cyp17* showed completely opposite behavior in the polar and non-polar extract treatments. Estradiol levels were significantly higher in the cell treated with the non-polar extracts at all concentrations. Elutriate exposure led to less pronounced variations with regard to both mRNA levels and hormone synthesis. Overall the extract with non-polar compounds induced the most dramatic variation in H295R cells and by consequence, most probably, the direct ingestion of detritus and mud from fishes and other benthonic organisms in the area, and consequent possible transfer throughout the trophic web, is mainly accountable for the alteration of the endocrine system previously observed for fish such as Tilapia species in the study site.

**101 Effects of sediments spiked with low fluoranthene concentrations on juvenile prawns from Tecolulta, Veracruz, Mexico**

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Polycyclic aromatic hydrocarbons (PAH) are common pollutants in aquatic environments because of their hydrophobic nature, they are deposited in sediments where they can be persistent. Fluoranthene (Fl) is considered a model PAH; it is genotoxic, carcinogenic, mutagenic and teratogenic; so exposure to this compound can represent a serious environmental risk. The aim of this work was to evaluate the effects of sediments contaminated with low fluoranthene concentrations (> EPA SQC) on juvenile prawns of the genus *Macrobrachium*. Sediments and organisms were collected in the Tecolutla estuary (Veracruz, Mexico) and transported to Mexico City in 20 L container. While the prawns acclimated for a month to laboratory conditions, sediments were dried, sieved and spiked to prepare a control and two concentrations: 225 and 450 µgFl/gOC. A 21day bioassay was carried out, with 25 organisms (average length 5 cm; 1 g of wet weight ) per concentration. During the bioassay, the following parameters were monitored and evaluated: oxygen:nitrogen ratio (O:N), lysosomal stability and swimming resistance, these evaluations were carried out on days 7, 11, 18 and 21. Swimming resistance decreased as time passed, the control group showed the highest values (average 50 sec), followed by prawns exposed to 225 µgFl/gOC and finally those in 450 µgFl/gOC (14 sec on day 21). The same pattern was observed in the O:N ratio (with average O:N of 98, 28 y 21 respectively) and the retention time in the lysosomal stability test (180 min, 120 min and 60 min respectively on day 21). In conclusion fluoranthene concentrations below the EPA SQC did not cause mortality but the physiological effects observed in juvenile prawns indicated that they are stressed and at a higher risk of being predated, which could lower their survival chances.

**102 State of the Science of Mayfly Ecotoxicity Testing**

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The historical choice of invertebrate species used in most current water and sediment toxicity test protocols represents a combination of practical (*e.g.*, amenity to year-round laboratory culturing, ease of maintenance, and regulatory acceptability) and ecological (*e.g.*, geographic and habitat representation, feeding habits, and relative sensitivity) considerations. This has led to the development of widely applied toxicity test protocols that incorporate reliable, though not necessarily highly sensitive test species. Early exploration of candidate species for toxicity testing included evaluation of EPT (Ephemeroptera, Plecoptera, Trichoptera) taxa. However, many of these were found to be difficult to culture (*e.g.*, many EPT taxa require flowing water), biological requirements were not well known, most would not reproduce under laboratory conditions, and many were sensitive to the laboratory environment (often failing to meet control test acceptability criteria during tests). Such early efforts with EPT species were therefore largely abandoned in favor of more pragmatic species (e.g., *Daphnia magna*, *Ceriodaphnia dubia*, *Hyalella azteca*, *Chironomus dilutus*/*riparius*) now used in most of the standard toxicity test protocols. However, field and laboratory studies have consistently shown that the EPT group of insects contain some of the most sensitive species to environmental contaminants. In recognition of recent advances in culturing and application in aqueous and sediment-based toxicity testing of EPT species, a project was initiated to report on the state of the science for the application of mayflies in ecotoxicity testing. The first part of the project involved a literature search for peer-reviewed and grey literature on Ephemeroptera species that covers their ecology, species' ranges, relative sensitivity among mayfly species used in toxicity tests, and a comparison of the sensitivity of mayflies to standard aquatic toxicity test species. Key goals of the project include the identification and prioritization of knowledge gaps that can serve as areas for future research, the assessment of the prospect for routine incorporation of mayflies in toxicity testing, and the creation of standardized toxicity test protocols. *Disclaimer: This presentation does not necessarily reflect the views or the policies of the U.S. Environmental Protection Agency.*

**103 Evaluating the Relative Sensitivity of the "P&T" in EPT: Implications for Standardized Toxicity Testing**

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The EPT (Ephemeroptera, Plecoptera, and Trichoptera) group of insects are widely applied as indicators of water quality in biomonitoring because they contain some of the most sensitive species to environmental pollutants. This widespread application in the field has not translated into standardized lab-based toxicity tests, in part, because many of the taxa tested in the early days of test development were difficult to culture due to unique environmental requirements (e.g., flowing water), most would not reproduce under laboratory conditions, and some were sensitive to the lab environment (often failing to meet control test acceptability criteria). In the past few years, however, interest in developing standard toxicity test protocols using EPT taxa has been renewed due to improved methods for culturing, development of novel exposure systems for obligate flowing water species, and an improved understanding of biological requirements. To date, much of this effort has focused on European and North American species of Ephemeroptera (mayflies) but recent testing with both Plecoptera (stoneflies) and Trichoptera (caddisflies) with neonicotinoid insecticides indicates that standardized exposure protocols may be possible. In this presentation, we will review historical and recent applications of Plecoptera and Trichoptera species in toxicity testing with the goal of evaluating their suitability for lab-based chemical assessments. In addition to practical considerations, we examined the relative sensitivity of EPT taxa based on studies derived from the literature and the application of Species Sensitivity Distributions developed for hazard assessments with neonicotinoid insecticides. We show that Ephemeropteran species, on average, are more sensitive than Plecopteran and Trichopteran species but that there is wide variation within and between the orders depending on genus, life history characteristics, and chemical class.

**104 Improving Sediment Toxicity Test Interpretation at Mega Sites Using Novel Approaches to Batch Normalization**

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Sediment toxicity tests are an important tool used in ecological risk assessments for contaminated sediment sites, but interpreting test results and defining toxicity is often a challenge. This is particularly true at mega-sites where a large testing regime necessitates performing bioassays in a sequence of batches, each evaluating a subset of the samples. Batched testing introduces variability and uncertainty that add to the challenge of interpreting results. Accounting for variability introduced by batched data collection is important. The absolute performance of test organisms is influenced by multiple factors, which contributes to variation among batches of sediment toxicity tests. These factors include the starting size, age, and health of the organisms used, conditions within each test, and random variation. For analyses that incorporate data from multiple batches of sediment exposures, the presumed goal is to parse differences in organism performance between batch-related variation and contamination-induced changes, such that the overall exposure-response gradient can be evaluated independent of batch-related variation. Variation among batches is often addressed by normalizing test sample results to control sample results, but this approach has potential for creating artifacts in the analysis. Alternative approaches include, for example, normalizing the results in each batch to a fixed percentile of the response distribution of each batch, and normalizing test results to the median (or other statistic) response in each batch associated with reference samples or with site samples determined sufficiently uncontaminated that no substantive biological response is expected. We used simulated bioassay data sets generated by resampling from log-logistic concentration response models (based on the results from real bioassays), to compare results among normalization procedures. The advantage of using simulated data was that we could separately evaluate the effectiveness of normalization approaches under specific batch variation scenarios (e.g., reduced control performance relative to test samples in some batches). The results of the simulation study illustrate the unintended results that can occur with control normalization, and the potential advantages of alternative approaches. However, there are assumptions implicit to each approach, and the most suitable approach may depend on specific characteristics of the bioassay data set.

## Wildlife Ecotoxicology Supporting Management Decision Making

**105 Characterizing risks to wildlife: Proposals for improving laboratory protocols and integration of alternative lines of evidence**

*M.S. Johnson, US Army Public Health Center / Toxicology; B.E. Sample, Ecological Risk, Inc.*

Predicting risk to wildlife species from exposures to chemicals requires an understanding of class-specific toxicology and variation of sensitivity within a class. Few laboratory wildlife models exist and there are few examples of controlled laboratory study designs that support toxicity reference value derivation. Incomplete toxicity data sets, variation in response, differences in study conduct, and statistical error all contribute to variations in interpretation. For example, differences between species might be due to variation in toxicokinetics driven by environmentally weathered forms, differences in gastrointestinal physiologies, or differences in exposure characteristics. Toxicodynamic differences within a vertebrate class also have the potential to affect toxicologic sensitivity and affects the predictive capabilities of risk assessments. Here we present some of the challenges and sources for variation in predictive toxicity benchmarks and provide some solutions for higher tiered post hoc risk assessments. In addition, some suggestions for toxicology protocol development are also presented to help improve predictive utility of toxicity-based benchmarks.

**106 What Do Tissue Residues Tell Us About the Utility of Body Weight Scaling for Interspecies Extrapolations in Wildlife?**

*P.C. Fuchsman, L. Brown, A. O'Connor, M.H. Henning, Ramboll*

Differences among bird or mammal species' responses to contaminant exposures reflect differences in toxicokinetics (where chemicals go after being taken into the body) and toxicodynamics (how chemicals interact with the site of toxic action). An important factor affecting interspecies toxicokinetic differences is metabolic rate, which is a function of body weight. Bioaccumulation takes longer to reach equilibrium in larger animals, and equilibrium concentrations in tissues are higher, due to slower metabolism compared to small animals. During the 1990s, ecological risk assessors used a body weight scaling approach to account for this phenomenon, for purposes of extrapolating toxicity data among species with different body weights. Efforts were made at that time to determine chemical-specific scaling factors through comparisons of acute toxicity test results among species, but this effort produced implausible results, likely because the internal exposures of larger species had not reached equilibrium during the acute tests. In 2007, a group of leading wildlife risk assessors concluded that in the absence of sufficient multispecies chronic toxicity data sets, body weight scaling should be dropped from ecological risk assessment practice. On the other hand, in 2011, the U.S. Environmental Protection Agency extended the use of body weight scaling in human health risk assessment, for purposes of determining reference doses from animal test results. We suggest that multispecies data sets from chronic bioaccumulation studies provide a potentially useful line of evidence to address the current lack of coherence in interspecies extrapolation practices. As case studies, we examine extensive laboratory and field data for avian bioaccumulation of cadmium, mercury, and PCBs. We compare how well different measures of exposure account for interspecies variation in the relationship between external and internal contaminant exposures. Exposure metrics include dietary concentrations, doses (as mg chemical per kg body weight per day), and mallard-equivalent doses (determined by body weight scaling). Our findings suggest it may be time to rethink the current practice of extrapolating toxicity data among species on a dose basis without accounting for differences in body weight.

**107 Field Studies to Support Management of Molybdenum Exposure to Mule Deer and Elk at a Molybdenum Mine in New Mexico**

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Molybdenum (Mo) mine tailings from a mine in New Mexico were deposited in the Tailing Facility (TF). Closure of the TF requires the replacement of the current variable soil cover and establishment of a native plant community. Mule deer and elk that currently forage on the TF may be exposed to Mo in tailings through plant bioaccumulation. To minimize potential exposure, plants selected for the future final cover should minimize both Mo bioaccumulation and preference as forage for deer and elk. Diet composition of deer and elk currently grazing on the TF was determined over four seasons using plant chloroplast DNA identified in scat samples and quantified by comparing scat DNA to the site-specific DNA reference library and to a more comprehensive DNA database from the European Molecular Biology Laboratory. Bioaccumulation was described using both loglinear regression and bioaccumulation factors (BAF) for co-located soil and foliage samples for 17 plant species (14 in and 3 not in diets). Although plant species consumed overlap, diets of elk and deer vary relative to each other and seasonally. Diet data strongly indicate substantial portions of diets are obtained off-site. Most common species growing on TF in the diet of both species were prickly lettuce, yellow sweet clover, alfalfa, hairy false goldenaster , common sunflower , hoary tansyaster, ragleaf bahia, and big sagebrush. Mo bioaccumulation rates vary by plant species. Both elk and deer at the TF displayed higher preferences for plant species that accumulated greater Mo. The 4 species that accumulated the greatest Mo (yellow sweetclover, alfalfa, ragleaf bahia, and hairy goldaster) were ranked 1, 2, 3, and 5 and 1, 2, 3, and 4 in diet preference for elk and deer, respectively. Mean Cu:Mo ratios were below PNEC of 1.3 for all 17 plant species evaluated, suggesting potential for toxicity. Of 6 plants with the lowest Cu:Mo ratios, only western wheatgrass was not consumed by elk or deer. The remaining 5 species, ordered by increasing Cu:Mo ratios, are Indian ricegrass, smooth brome, yellow sweetclover, alfalfa, and needle and thread grass. Based on these results, 6 species (rubber rabbitbrush, blue grama, bottlebrush squirreltail, big sagebrush, western wheatgrass, and common sunflower) should be considered for re-vegetation seeding mix. Alfalfa and yellow sweetclover, should be excluded due to their forage preference, high molybdenum bioaccumulation rates and low Cu:Mo ratios.

**108 Should ERAs Include Mammals? Tales from a Gray Fox Study**

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Ecological risk assessments (ERAs) should only evaluate those species that are spatially relevant to their contaminated environs. To that end, ERA practitioners should seek out opportunities to establish that species they may believe to be of interest, actually satisfy a key, but often overlooked receptor-of-concern selection criterion, namely a demonstrated high site fidelity. The use of newly available, state-of-the-art GPS tracking equipment to investigate the worthiness of gray fox (*Urocyon cinereoargenteus*) inclusion in terrestrial assessments, brings forward exposure information of great value. As with the outcome of a similar spatial movements tracking effort for White-tailed deer (presented at a SETAC N.A. meeting a number of years ago), gray fox will probably never be appropriate for consideration within an ERA setting. The high-quality actual, as opposed to modeled, exposure data of this study constitutes a classical example for wildlife ecotoxicology information supporting management decision-making. The specific data presented contributes to a growing and persuasive argument that mammal inclusion in ERAs for Superfund-type sites is quite unnecessary.

**109 The Use of Geographic Information Systems (GIS) for Spatial Ecological Risk Assessments: An example from the Athabasca Oil Sands area in Canada**

*K. Eccles, University of Ottawa / Department of Biology; B. Pauli, Environment and Climate Change Canada / National Wildlife Research Centre; L. Chan, University of Ottawa / Biology*

Modelling impacts of chemical mixture exposures across a landscape is challenging due to the spatial heterogeneity and complexity of the sources, pathways, and fate of the multiple chemicals in the mixture. This is especially true for estimating exposure of wildlife living in areas of intense anthropogenic disturbance such as the Athabasca Oil Sands area in Canada. In the field of ecotoxicology there is an acknowledged need to include spatial analyses to develop more accurate risk assessment. As a result, the field of landscape ecotoxicology emerged, which studies the spatial distribution of contaminants across a landscape and the impact on ecological systems. While landscape ecotoxicology and spatial analyses can theoretically be useful for risk assessment purposes, they have yet to become common practice due to the underdevelopment or standardization of methodology. Here, we propose and demonstrate the use of geographic information systems (GIS) as a tool to integrate data, assess large spatial scale patterns across contaminants and species, and assess complex exposures for landscape ecotoxicological assessment using data collected under the Joint Oil Sands Monitoring (JOSM) project in the Athabasca Oil Sands area. This dataset is comprised of 1100 biological samples from five individual biomonitoring projects on tree swallows, amphibians, gull and tern eggs, plants, and terrestrial and semi-aquatic mammals. The samples were analyzed for a variety of contaminants including metals and polycyclic aromatic compounds (PACs), and biological health endpoints. The data were spatially combined and mathematically integrated in ArcGIS 10.4 and analyzed using spatial methods such as Getis-Ord's Gi*, Moran's I, and spatial densities to assess landscape level spatial patterns. The results and spatial patterns are intuitively communicated through maps. Our results demonstrate that GIS models can be used in spatial ecological risk assessment and in developing evidence-based long-term ecological monitoring programs.

**110 Population Viability Management to Inform California Condor Conservation**

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Population variability analysis (PVA) has long been recognized as a valuable tool to assess population status and trends in wild species. However, the integration of toxicology data into PVA models is still relatively rare. Population viability management (PVM) models incorporate management into the PVA structure in order to predict the effects of alternative management actions on population stability and growth. We stable lead as well as carbon and nitrogen isotopic analyses to demonstrate that ingestion of spent lead ammunition from feeding on carcasses was the principal source of lead to free-flying California condors (*Gymnogyps californianus*) in California and that condors were exposed to DDE from feeding on marine mammals. We then constructed a PVM for condors to quantify the effects of contaminant exposure (e.g., lead, DDE) and multiple management actions on population growth. We found that lead poisoning is the primary threat preventing condor recovery in the wild. Our published results of this work informed legislation, leading the California legislature to pass and Governor Brown to sign into law in 2013 a ban on lead-based ammunition for hunting within the state of California that will be fully implemented by 2019. Currently, we are using PVM to prioritize management actions for the effective use of limited management resources to enhance condor population growth under different conditions, such as mortality from lead poisoning and reduced reproduction from DDE. Adult condor survival is the most important factor for population growth and lead is the main threat affecting adult survival. Consistent with this, our analyses found that nest management, which increases chick survival, is less important to long-term (e.g., 30-year) population growth than releasing captive-bred condors. We have also shown that establishing a new condor release site, which will reduce the number of available captive-bred condors for release to existing flocks, decreases the population growth rate of condors in California, though this effect can be mitigated by increasing the number of captive-bred birds available for release into the wild. Our findings demonstrate how toxicology data and management practices can be used in a PVM framework to provide important quantitative predictions on population health to inform policy and management.

**111 Challenges in Monitoring Anticoagulant Rodenticides in Wildlife in California**

*S. McMillin, CA Dept of Fish and Wildlife*

The California Department of Fish and Wildlife (CDFW) has been monitoring anticoagulant rodenticide exposure in wildlife since the 1990s. These findings were used by the California Department of Pesticide Regulation to designate second-generation anticoagulant rodenticides California restricted use materials in 2014. CDFW has continued this monitoring project to evaluate effectiveness of the regulation change. Interpretation of these data is challenging for a number of reasons. First, inherent biases in sample collection are difficult to avoid. For example, most of the carcasses submitted for analysis are collected by wildlife rehabilitation centers, usually located near populous areas of the state. However, some studies have taken advantage of opportunities for less biased sampling, such as the statewide mountain lion study, the Bakersfield San Joaquin kit fox study, and the fisher study. Second, careful interpretation of both pesticide residue and clinical findings is required. Differences in species sensitivity to anticoagulants and in toxic effects of different active ingredients, as well as changing reporting limits, may complicate interpretation of quantitative findings. Also, co-occurring mortality factors such as trauma and disease, must be considered when making conclusions from necropsy findings. Finally, exposure circumstances can be difficult to determine. Many predators and scavengers have large ranges so locations of exposure are unknown. Additionally, for most submissions it is impossible to determine if clinical toxicity cases resulted from a single exposure event or multiple exposures. Information about dietary sources of exposure is lacking, especially for species with diverse diets. While these factors must be considered when attempting to describe effects of anticoagulant rodenticides on wildlife, some conclusions can be made from the data, including continued exposure of a wide range of wildlife species distributed throughout the state three years after regulation change and continued cases of intoxication from these exposures.

**112 Wildlife Ecotoxicology in California Natural Resource Damage Assessments**

*C. Marn, US FWS / San Francisco Bay-Delta Fish and Wildlife Office*

When wildlife and other natural resources are injured as a result of the release of hazardous substances or the discharge of oil into navigable waters, federal, state, and tribal governments in the United States may act on behalf of the public to seek compensation from responsible parties to restore injured resources. Natural resource trustees have the authority under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) and the Oil Pollution Act (OPA) to conduct natural resource damage assessments (NRDA) to assess injuries to natural resources and quantify monetary damages. Damages recovered are used to restore the injured resources and compensate for lost services. As defined in CERCLA and OPA, NRDA is a process to evaluate the nature and extent of injuries to natural resources and services resulting from the release/spill and to determine the restoration actions needed to recover injured resources back to the baseline conditions that would have existed had the release or spill not occurred. Natural resource trustees must determine whether injuries have resulted from the release/spill and quantify the degree, and spatial and temporal extent of the injuries relative to baseline before developing restoration alternatives. In support of the claim for restoration, natural resource trustees may use data from field studies, laboratory studies, modeling, and published literature. Examples of data collected from California NRDA cases, specifically related to assessment of injuries to wildlife and other biota will be presented to illustrate the NRDA process.

## The Need for Data and Data Management Tools to Address ESA-listed Species Protection and Agricultural Production Goals

**113 Exploring Complementary Options that Optimize the FIFRA/ESA Process**

*L. Duzy, A.R. Frank, B.D. McGaughey, Compliance Services International; T. Carro, P. Whatling, FMC Corporation*

Under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), the process of assessing the potential impact of pesticide use on species listed under the Endangered Species Act (ESA) can be resource intensive for all stakeholders. In order to address risk management and species protection, there is interest in exploring alternatives or complements to the current process. Boyd's OODA Loop is applied to the process of building options. One option is to frame and evaluate risk and uncertainty given best available data to evaluate reasonable and affordable voluntary mitigation or management options, such as targeted conservation to effectively protect and enhance listed species and their habitats while efficiently bringing new products and product uses through the registration and consultation process. This presentation will introduce the exploratory efforts underway to understand and combine use site, species, product, and economic factors in a way that builds a basis for development of strategies to achieve effective and protective registration actions.

**114 Integrating multiple sources of data to characterize the risk of contaminants to ESA-listed species; Part I – visually consolidating data**

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Assessing the risk posed to an ESA-listed species by contaminant exposures requires integrating numerous types of information. Much of the information will be species-specific and vary with time, space, and habitat. A given contaminant, for example, can arise from different sources and enter different habitats for varying times. Even for a single species, therefore, there can be numerous, different potential exposure concentrations each with their own uncertainty or variability. Comparing the locations of the sources of exposure to species range and life-history information is also important. Finally, numerous species-specific toxicity endpoints for the contaminant need to be considered. Examples include both lethal and sublethal effects (e.g. mortality, growth and behavior) as well as habitat-mediated effects (e.g. impacts on prey abundance). This presentation will summarize an approach to gathering the relevant data for a species into a single plot designed to help characterize the risk posed by a contaminant (an “R-Plot”). The current tool is based on R and automates the generation of a plot that displays these multiple sources of data in one figure. An example will be presented for the risk posed by a pesticide to an aquatic species.

**115 Integrating multiple sources of data to characterize the risk of contaminants to ESA-listed species; Part II-application to pesticide consultation**

*R.J. DeWitt, The Evergreen State College / Office of Protected Resources Endangered Species Division; D.H. Baldwin, NOAA Fisheries / Northwest Fisheries Science Center; T. Hooper, NOAA Fisheries / Office of Protected Resources Endangered Species Division; C.A. Laetz, NOAA /NMFS / Northwest Fisheries Science Center; S.A. Hecht, NOAA / National Marine Fisheries Service; T. Hawkes, National Marine Fisheries Service / Office of Protect Resources*

Evaluating the effects of EPA’s nationwide authorization of pesticides to federally-listed threatened and endangered species requires integrating numerous types of information including species-specific life-history information; exposure estimates that vary with time, space, and habitat type; and multiple types of effects data relevant to a species fitness including sublethal effects, lethal effects, and habitat-mediated effects. In a recent biological opinion, National Marine Fisheries Service developed an approach for gathering relevant data for a species into a single plot (an “R-Plot”). This presentation will describe how R-Plots were used to inform the effect, likelihood, and confidence of pesticide exposures to aquatic ESA-listed species. In addition, how data were selected and how R-Plots were utilized to assign low, medium and high rankings to pesticide use categories will be described. Finally, an overview will be provided on how the resulting rankings were then integrated into the overall assessment of risk to populations of federally-listed species and their designated critical habitats.

**116 FIFRA/ESA Assessments and Beyond: How FESTF’s Data Management Tool, Gopher, Answers Questions and Provides Options**

*A.R. Frank, Compliance Services International; T. Hall, Bayer / Environmental Toxicology and Risk Assessment; D. Campbell, Syngenta / Department of Regulatory Affairs; B.D. McGaughey, Compliance Services International*

The 2017 release of the US Environmental Protection Agency’s (USEPA) first Biological Evaluations (BE) under Registration Review to include national-level endangered species assessments, and subsequently the 2018 Biological Opinion (BO) from the National Marine Fisheries Service (NMFS), illustrated some of the challenges of balancing pesticide registration decisions with agricultural production and endangered species protection objectives. Specifically, the limited availability of pesticide use data, refined species distribution information, and the lack of a data management tool to aggregate and analyze such data made it difficult to adequately consider both objectives. The FIFRA Endangered Species Task Force (FESTF) has contributed to the US Environmental Protection Agency’s (USEPA) endangered species and pesticide data development process for nearly 20 years and has recently completed an enhanced service tool – Gopher – that integrates best available datasets related to pesticides and endangered species into a single tool. Using examples from the BE and the BO, this presentation will illustrate how Gopher can be used to manage, synthesize, spatially interact with, and retrieve data to bring efficiency to the process. Future enhancements of the system will be discussed including ways to better inform Environmental Baselines with incorporation of pesticide use data and capturing on-the-ground conservation efforts.

**117 My Compliance Program has a Weed Problem: Managing Aquatic Plants in the Sacramento-San Joaquin River Delta**

*J. Madsen, P. Moran, P. Pratt, USDA / ARS; E. Hard, California; J. Caudill, California State Parks / Division of Boating and Waterways; W. Pratt, Crowe Horwath; D. Bubenheim, NASA / Ames Research Center*

The Sacramento-San Joaquin River Delta (“Delta”) is the largest freshwater estuary on the West Coast, and provides critical water resources, ecosystem services, and habitat to 56 rare, threatened, or endangered species. Water resource provision and species habitats are imperiled by infestations of invasive aquatic plants, including waterhyacinth, Brazilian waterweed, and waterprimrose; which obstruct water supply, navigation, and endangered species survival. Environmental regulatory requirements affect all aspects and activities in managing these species, from planning through implementation, site selection, and setbacks. Compliance with federal Endangered Species Act has resulted in pesticide and surfactant testing for all life history stages and food chain trophic levels for Delta smelt and pacific salmonids, as well as additional oversight and setbacks for giant garter snake and valley elderberry longhorn beetle habitats. While these additions have potentially decreased risk of harm to endangered species, the cost to successfully manage the invasive plant problem has increased dramatically while reducing the available tools. Much of the floating aquatic vegetation management must either be delayed or suspended to accommodate timing and setback requirements, submersed plant treatments have to utilize repeated treatments of a more expensive alternative in lieu of a less expensive herbicide. Despite these obstacles, management of waterhyacinth in 2017 managed to keep up with growth after the explosive growth in 2014, 2015, and 2016. Intensive research and development activity has resulted in the introduction of herbicides and surfactants with lower risks to species of concern. Research by USDA ARS has led to the release of new biological control agents for waterhyacinth and giant reed. A decision support tool has been developed to provide acreage of waterhyacinth and waterprimrose estimates from LandSat data on a biweekly basis, increasing the ability to locate nursery populations of these weeds. The management of invasive aquatic plants has been recognized as a critical component to the recovery of Delta smelt populations in the Delta. An adaptive integrated pest management approach has been developed for effective management of invasive aquatic plants that fully considers the environmental impacts on endangered species and environmental compliance of with a full range of regulatory concerns.

**118 Methods for Generating Best Available Agricultural Land Use Data in Support of Pesticide Ecological Risk Assessments**

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The USEPA and partner agencies are required to assess all registered pesticide uses for their potential impacts on all species listed under the

Endangered Species Act (ESA). In the context of the risk assessment process it is imperative to identify data sets that have a high degree of accuracy in order minimize the amount of uncertainty in the risk estimates and to avoid relying on assumptions that could be excessively conservative. The importance of utilizing land use data and pesticide use data that have a high degree of accuracy is one example that cannot be overstated because they are both directly involved in estimating the risk associated with specific pesticide uses. Because mandatory pesticide use reporting data is scarce, risk assessors rely on the best available data and modeling efforts. In 2002, the Washington State Department of Agriculture (WSDA) began developing a statewide agricultural land use mapping program to generate data utilizing GIS technologies that would be able to provide highly accurate land use data for the purpose of informing pesticide's risk assessments for endangered species. Mapping programs like the one developed by WSDA are especially important because standard remote sensing technology is more problematic in areas where there are a large variety of crops like Washington State which has over 300 different crop types. The geospatial dataset is published to a geodatabase where the data is then accessible via web maps and mobile apps and can be used quantitatively by researchers and risk assessors as well as a wide range of other user groups. Staff involved with the data collection at WSDA also work closely with the United States Department of Agriculture (USDA) and participates in the Cropland Data Layer (CDL) project by providing Washington State specific ground-truthed data for their satellite product. Participation in the CDL project ensures that the data representing Washington State in the CDL is as accurate as possible and is available for use for national assessments by federal agencies.

**119 Managing pesticides and the watersheds of Washington State for the protection of ESA species, and economic vitality**

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The watersheds of Washington State are managed by multiple state, federal and tribal agencies to ensure multiple ecosystem services. Agricultural productivity in the Skagit, Nooksack and Yakima watersheds are vital to the economy of the state. Chinook salmon are an iconic species of the Northwest and the evolutionary significant units are protected by the Endangered Species Act. The runs of Chinook Salmon are also of cultural importance to the Tribes and to many of the cultural groups in the region. The salmon runs are also economic events. The Skagit Eagle Festival in Concrete, WA and along the watershed in the winter is a major tourist event with thousands of Bald Eagles drawn by the runs of the Chinook salmon. In the Yakima River dams are present to supply irrigation and pesticides are applied to assure the agricultural productivity of the region. However, the dams are barriers to passage for Chinook passage, isolating some salmon populations. Pesticides are toxic to the fish and to the necessary invertebrate assemblages. Across the State global climate change is altering water temperature, the type of precipitation during winter, the timing of flood events and ocean acidification is lowering the pH of near shore marine waters. A quantitative framework is required to evaluate the effects of multiple stressors on the multiple ecosystem services provided by the vital watersheds across the Puget Sound and Columbia watersheds. Bayesian network risk assessments have been built to describe the interactions of organophosphate pesticides, in-stream dissolved oxygen and temperature, land use, changes in pH, frequency of storm events and how they affect a wide variety of ecosystem services. We will describe how these efforts can be integrated into Bayesian network relative risk models that include utility nodes. These constructs can be used to describe the trade-offs of different management options in a way to inform adaptive management.

**120 PRESCRIBE: Online Database and Mobile Application for protection of endangered species from pesticides**

*L. Moreno, C.A. Bilheimer, California Dept. of Pesticide Regulation / Endangered Species Program*

The California Department of Pesticide Regulation (DPR) has worked with local stakeholders to develop an endangered species protection program that is comprehensive in scope and includes all federal- and state-listed species and all pesticides registered in California. The core of DPR's program is a free, Web-based database application that allows pesticide applicators and others to identify local habitat for endangered animals and plants, and advises applicators on required use limitations when necessary. The system is called PRESCRIBE, acronym for "Pesticide Regulations Endangered Species Custom Real-time Internet Bulletin Engine." PRESCRIBE (http://cdpr.ca.gov/docd/es/prescint.htm) contains location records for over 1,000 endangered, threatened, and other special status species, encompassing almost 80,000 unique land sections. It also can search for 30,000 pesticides by brand name. In a PRESCRIBE query the user selects the county-township-range-section(s) where he/she intends to apply pesticides and then the pesticide intended for use. The database application looks up the species that occur in the selected section(s) and the active ingredients in the selected pesticides and generates a report of what listed species occur in the area and what use limitations may apply to the selected pesticides for protection of those species. These use limitations were derived from biological opinions developed by the U.S. Fish and Wildlife Service (USFWS) in consultation with the U.S. Environmental Protection Agency (USEPA) and reviewed by USFWS. The endangered species section-habitat index is derived from the Department of Fish and Wildlife (DFW) Natural Diversity Database, as well as maps obtained from USFWS and National Marine Fisheries Service (NMFS). The use limitations are voluntary, unless required by pesticide labeling or pesticide use permits. At the same time, DPR provides pesticide users with training that includes educational materials to help them identify endangered species habitats and pesticide-specific use limitations. PRESCRIBE Mobile, a new mobile application (https://mobile.cdpr.ca.gov/prescribe) also yields protective measures and adds a feature that uses a smart phone's geographic locator function to identify a user's current location and proximity to listed species' habitat.

## Epigenetics and Environmental Exposures: Mechanisms and Effects from Invertebrates to Fishes

**121 Oil and hypoxia induced alterations to DNA methylation patterns in developing sheepshead minnows (*Cyprinodon variegatus*)**

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It is increasingly evident that early-life stress alters the establishment of epigenetic marks. In particular, developmental exposure to toxicants or adverse environmental conditions can alter normal patterns of DNA methylation and have persisting effects on the regulation of gene expression. However, the extent to which these factors interact to modify epigenetic programming remains largely unknown. During the 2010 Deepwater Horizon (DWH) oil spill larval estuarine fish were simultaneously exposed to both polycyclic aromatic hydrocarbon (PAH) contaminants and hypoxia. We investigated how these stressors act individually and in combination to modulate the establishment of DNA methylation marks in developing sheepshead minnows (*Cyprinodon variegatus*). We exposed larval sheepshead minnows (4 days post-hatch) to oil, hypoxia, or both for 48 hours followed by 48 hours of depuration in clean, normoxic conditions. DNA immunoprecipitation coupled with high-throughput sequencing (MeDip seq) was used to identify differentially methylated genes among treatments. We performed pathway analysis on identified genes to predict the biological impacts of differential methylation and compared the results of our methylation analysis with

RNAseq to examine the relationship between promoter methylation and gene expression patterns. This is the first study to examine DNA methylation changes induced by the combination of exposure to DWH oil and environmental stressors. Because DNA methylation patterns can persist throughout the lifetime of an organism, our data provide insight into whether oil-induced changes in gene expression might be permanent due to alteration of epigenetic profiles, and will enhance our understanding of the long-term individual and population level effects of oil and hypoxia exposure.

**122 Deciphering the epigenetics mechanisms underlying the transgenerational bone toxicity of Benzo[a]pyrene**

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Recent studies have raised the awareness that Benzo[a]pyrene (BaP) is an transgenerational toxicant with the potential to affect fish bone tissue integrity in the unexposed offspring (< F3). Using the medaka, a fish model established for human skeletal diseases, we demonstrated that ancestral exposure to environmentally relevant levels of BaP resulted in vertebral deformity in the F1-F4 descendants. Increased occurrence of dorsal-ventral compression of vertebra segments was found in the unexposed F1-F4 larvae. A decrease in bone thickness was persistent in the adult F3and F4 BaP lineage. The use of the col10:gfp/osx:mcherry transgenic medaka fish and histological tissue analysis revealed a reduction of osteoblast abundance in the F1-F3 larvae. Deregulated expression of osteogenic key genes pointed towards an impairment of osteoblast differentiation (larvae) and osteoblast activity (adult), which might be causative for an increased bone fragility. BaP-induced epigenetic changes were evident by significant changes in the immunohistochemical expression of histone methylation (H3K4me2, H3K27me2) in bone lining cells as well as the global DNA methylation patterns in F3 larvae and F2 sperm (BaP vs CTL). The antagonistic expression of conserved bone miRNAs with their target bone genes in F3/F4 vertebrae (by qRT-PCR) led to the identification of five functional pairs of mRNA/miRNA in the ancestral BaP exposed medaka. The results shed light on the potential target genes for BaP-mediated epigenetic inheritance, which warrant further investigation. From an ecotoxicological perspective, abnormal skeletal integrity renders the fish more susceptible to bone fractures and exerts a far-reaching impact on individual fitness and survival. Re-assessment of the ecological risk of BaP is urgently needed. The molecular pathways for bone formation and metabolism are highly conserved in vertebrates. The results obtained in the medaka model may allow extrapolation on the potential transgenerational epigenetic mechanisms of BaP on skeletal disorders in other vertebrates, including mammals.

**123 Differential Expression of microRNAs in Fathead Minnow Larvae Exposed to Ethinyl Estradiol**

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Reduction of uncertainty in stressor-induced exposure assessments relies on characterization of a number of molecular processes that are easily measurable, can be integrated with other process indicators, and ultimately relate to changes in apical endpoints. In both human and ecological model organisms, study of changes in expression of mRNA transcripts has dominated this characterization of molecular processes. However, microRNAs (miRNAs), which are small non-coding RNA molecules, function in RNA silencing and post-transcriptional regulation of gene expression and offer another, potentially toxicologically integratable, window into exposure- and effect-related molecular pathways. Here, methods for analysis of small ribonucleic acids, 20-40 nucleotides in length (smRNA-seq) were used to study differential expression of miRNAs in fathead minnow (*Pimephales promelas*) larvae exposed to 10 ng/l 17a-ethinyl estradiol for 48 hours in static exposures (10 control; 10 exposed samples). The program miRDeep* was used with pooled smRNA reads and a very recent draft of the FHM assembly (n50 of ~300,000) to identify both known and novel miRNA species. Comparisons showed 122 miRNAs to be identical to miRNAs reported for *Danio rerio* reported at miRbase.org. Adapter-trimmed reads from individual samples were then mapped using Bowtie1 to the identified FHM miRNAs to yield sample feature counts. Feature counts were further filtered to yield sample count matrices with consistent levels of non-zero expression across samples. This will be among the first reports of differential miRNA expression in response to an environmental toxicant in *D. promelas*.

**124 Transgenerational effects of early life stage exposure to endocrine disruptors across biological scales in a euryhaline model fish**

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Emerging research demonstrates that endocrine disrupting compounds (EDCs), which agonize, antagonize, and / or synergize the effects of endogenous hormones, can cause deleterious effects in adulthood as a result of early-life exposure, as well as transgenerational effects. A paucity of studies exist in non-model species, such as *Menidia beryllina*, a euryhaline fish with short generation time that is found throughout North America and is demonstrated to be sensitive to contaminants. We exposed *Menidia beryllina* embryos (8 hpf) until 21 dph to environmentally relevant concentrations of an androgenic and estrogenic EDC of emerging concern: levonorgestrel (Levo) (10 ng/L), bifenthrin (Bif) (5 ng/L), respectively, and coupled this exposure with testing of an established androgenic and estrogenic EDC: trenbolone (TB) (10 ng/L), and ethinylestradiol (EE2) (5 ng/L), respectively. We evaluated the potential for transgenerational EDC effects in three generations across biological scales, with EDC exposure isolated to the parental generation (to 21 dph) only. We investigated changes in gene expression, DNA methylation, gonad histology, fecundity, sex ratio, morphology, and immune response. We also sequenced the *M. beryllina* genome using 10X genomics to facilitate DNA methylation analysis. F0 results show that early-life exposure to EE2 significantly skewed adult sex ratios (feminized) relative to controls. Findings from the F0 and F1 generations demonstrate that exposure to EDCs increased growth in the parental larvae, and that androgenic treatment groups (Levo, TB) maintain this growth through the subsequent F1 generation. In the F0 adults, differences in immune response are apparent between bifenthrin and levonorgestrel, and this pattern is stronger in F1 adults, with significantly greater T-cell proliferation in bifenthrin-exposed individuals relative to controls. Bifenthrin-exposed parental females have increased atretic follicles, and developmental defects are more pronounced in F1 embryos and larvae relative to parents. Transgenerational effects are evident in the unexposed F2 generation, with reduced survival in three treatments (Levo, TB, EE2), altered growth (EE2), and increased craniofacial deformities (Levo). Elucidation of the molecular mechanisms contributing to these higher order effects will inform adverse outcome pathways, as well as allow for the quantification and comparison of responses to established and emerging endocrine disruptors.

**125 Characterization of methylation changes and ERα gene expression as a result of EE2 exposure in fathead minnow liver**

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Estrogens present in the environment can interfere with endocrine function and cause decreased fecundity, fitness, and sperm production in fish, as well as feminization of male fish. Physiological effects and alterations of gene expression resulting from estrogen exposure have been thoroughly described in fish. Despite this, little is known about epigenetic alterations, although these changes are believed to provide the critical linkage of gene expression with the development of adverse effects at higher biological levels. This study investigates alterations of DNA methylation of the *estrogen receptor α* (*ERα*) in liver tissue in fathead minnows (*Pimephales promelas*) exposed to either 2.5 ng/L or 10 ng/L of the synthetic exogenous estrogen, 17α-ethynylestradiol (EE2). Methylation differences were assessed across all CpG sites in a 2.5KB region encompassing exon 1 and 1.5KB upstream of the start site of the *ERα* gene by targeted deep sequencing of bisulfite treated DNA isolated from treatment groups. Additionally, DNA methylation was assessed from fish depurated for 7 and 14 days to determine the kinetics of methylation. Finally, relationships between *ERα* methylation status and gene expression for individual fish were evaluated. Results from this work will provide information regarding the drivers of response to estrogens and the linkage between alterations in methylation status and gene expression.

**126 Transgenerational Inheritance of Atrazine-induced Reproductive Dysfunction by the Third Generation of Medaka (Oryzias latipes)**

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Epigenetic transgenerational inheritance of altered phenotypes are initiated in aquatic organisms by exposures to some endocrine disrupting chemicals. Currently, little is known about the mechanisms or the significance of such effects on wild populations. Atrazine exposure alters key hypothalamus-pituitary-gonad (HPG) endocrine pathways in fish, a potential mechanism for alteration of the epigenome during development. However, atrazine-induced transgenerational inheritance of reproductive effects in fish has not been investigated. The present study examined the effects of early developmental atrazine exposure on transgenerational reproductive dysregulation in Japanese medaka (*Oryzias latipes*). F0 medaka were exposed during the first twelve days of development (5 or 50 ug/L atrazine, 2 or 50 ng/L 17α-ethinylestradiol (EE2), or solvent control) with no subsequent exposure over three generations. This exposure overlapped with the critical developmental window period for embryonic germ cell development, gonadogenesis, and sex determination. Exposed males and females of the F0 treatment lineages were bred to produce the F1 generation, and this was continued through the F3 generation. Sperm parameters and gonadosomatic and hepatosomatic indices were unaffected in the F0 generation treatment lineages. Hepatosomatic index was reduced in F2 females from either EE2 or atrazine treatment lineages. Fecundity was unaffected by atrazine or EE2 in F0 through F2 generations; however, fertilization rate was decreased in low atrazine and EE2 treatment lineages in the F2 generation. There were significant transgenerational differences in expression of the genes involved in steroidogenesis and DNA methylation. Early life exposure to atrazine didn't cause significant adverse effects in the immediate generation (F0); however, alterations of HPG pathways may pose reproductive risks in future generations of fish.

**127 Developmental methylmercury induces epigenetic alterations in adult zebrafish and in future generations**

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Developmental exposure of zebrafish to MeHg alters their neurobehavior in a manner similar to other vertebrates. We investigated the direct and transgenerational effects of MeHg, at tissue doses similar to those detected in exposed human populations, on sperm epimutations (i.e., differential DNA methylation regions [DMRs]), neurobehavior (i.e., visual startle and spontaneous locomotion), and transcriptomics in zebrafish. F0 generation embryos were exposed to MeHg (0, 1, 3, 10, 30, and 100 nM) for 24 hours ex vivo. F0 populations were reared to adults and bred to yield the F1 generation lineages, which created the F2 generation lineages. Direct exposure (F0) and transgenerational actions (F2) resulted in hyperactivity and visual deficit in the unexposed descendants (F2) of the MeHg-exposed lineages compared to control. An increase in F2 sperm epimutations was observed relative to the F0 generation with DMRs in genes associated with neuroactive ligand-receptor interaction and actin-cytoskeleton pathways, which correlate to the observed neurobehavioral phenotypes. Developmental MeHg-induced epigenetic transgenerational inheritance of abnormal neurobehavior is correlated with sperm epimutations in F2 adult zebrafish. Further study of the transgenerational health effects of MeHg is required to better understand the risks for human and wildlife populations, which, if transgenerational effects are ignored, are dramatically underestimated.

**128 Consequences of cadmium-induced epigenomes on mutation, animal fitness, and adaptation**

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There is a pressing need to understand how environmental conditions, including toxicant exposure, influence genome content, structure, and function and, in turn, how individuals and populations cope with changing environments. It is now understood that genomes are more than static, heritable, biological templates, but rather display a wide range of plasticity that is modulated by the environment. These environmental perturbations of the genome are functionally important as we are quickly learning that they contribute significantly to variation in individual physiologies, fitness, dynamics of populations, and influence adverse outcomes. Both base-substitution and structural mutations are known to contribute to genome plasticity. While mutation is a stochastic event, mutation hotspots exist both within and between genomes. The genetic events that give rise to mutation (e.g., altered DNA damage/repair pathways, miscued replication, recombination error, transposable elements) are beginning to be defined in terms of their functions, yet little is known of the mechanisms that destabilize DNA, potentiate mutation, and coordinate mutation within the genome. In this talk, long term Daphnia mutation accumulation lines, which exclude selection, are used to test the hypothesis that environmental stress influences epigenetic states including histone modifications, which control access to the genome and affect organismal fitness by potentiating mutations. We report that cadmium induces histone modifications that signal active transcirption, shift nucleome occupancy, and change the distribution of mutations by increasing the their frequency within exons. Understanding these processes will have profound implications for society and the long-term health of populations, which are living longer in the presence of a large and growing diversity of chemicals that can modify DNA.

## Fate and Effects of Metals – Mechanisms of Bioavailability and Toxicity

### 129 Role of geochemical speciation and mineralogy on metal bioavailability

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The geochemical environment is a critical control on metal mobility and bioavailability when contaminants are released to freshwater systems. We show how integrating geochemical modeling, mineralogy, and bioavailability assessment using a model species in laboratory experiments can elucidate the relative importance of exposure pathways (aqueous vs dietary exposures) as well as the difference in bioavailability among solid phases such as metal sorbed to iron oxyhydroxides and pure mineral precipitates. Aqueous complexation with inorganic (e.g., carbonate) and organic (e.g., dissolved organic carbon) ligands can affect metal adsorption onto mineral surfaces as well as mineral solubility, controlling the distribution of metal between the aqueous and solid phase. Complexation also affects the bioavailability of metals to aquatic organisms. For instance, previous work has shown that carbonate complexation can limit the bioavailability of uranium in surface waters with moderate hardness. Geochemical modeling showed that >99% of dissolved uranium was complexed to inorganic carbon in a surface water with a moderate hardness. In addition, uranium complexation with dissolved organic matter has been shown to decrease the bioavailability from the aqueous phase, but had no detectable effect on uranium bioavailability from solid phases. A detailed understanding of geochemical speciation, mineralogy, and associations with dissolved organic matter is important to understanding the bioavailability of metals, including uranium, copper, zinc, and lead. Applying this integrated approach to complex environmental systems with elevated metal concentrations, such as lead-containing mill tailings, can advance our understanding of the geochemical controls on bioavailability.

### 130 Mercury species bioavailability and effects to green alga *Chlamydomonas reinhardtii*

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Based on the extensive research over the past 30 years, bioavailability is accepted as a key concept allowing to quantitatively relate the changes in the trace metal concentrations and speciation with the intensity of the induced biological effects. However, in the case of mercury the interactions at ambient medium-biota interfaces are not fully understood, and the interconnection between the mercury speciation, bioaccumulation and biological effects is still to explore. Present work focusses on the bioavailability of inorganic mercury (IHg) and monomethylmercury (MeHg) to model phytoplankton specie *Chlamydomonas reinhardtii*. The interactions of IHg and MeHg with green alga *C. reinhardtii* during short-term exposure to IHg (20pM to 100nM) and MeHg (10 pM to 50nM) were characterized by determining the amount of accumulated IHg and MeHg and the differential gene expression. Accumulated IHg and MeHg were determined w/o cysteine washing to assess the total and intracellular Hg contents. Results revealed a linear increase of the intracellular IHg and MeHg contents in *C. reinhardtii* with the exposure concentration range. The number of dysregulated transcripts determined by RNAseq raised proportionally to the increase of the intracellular Hg contents of both IHg and MeHg. However for the comparable intracellular content, the number of the dysregulated transcripts was higher for MeHg than for IHg, suggesting stronger impact of MeHg on algae as compared with IHg. At transcriptome level, exposure to MeHg dysregulated the expression of genes involved in motility, energy metabolism, lipid metabolism, and transport and antioxidant enzymes in *C. reinhardtii*, while IHg induced similar alterations but only at highest exposure concentration of 100nM. MeHg induced increase of the percentage of the cells experiencing oxidative stress, while no oxidative stress was detected for IHg exposure. The expression of several metal transporters' genes (e.g. Cu, Co, Zn) and aminoacids was affected by both species, finding that was in agreements with the performed competition experiments. The results of this basic research contribute to significant improvement of the understanding of Hg compounds uptake mechanisms and the adverse outcome pathways in green algae, a model of aquatic primary producers, as well as to the development of sensitive genetic biomarkers in support to biomonitoring efforts of mercury monitoring programs in aquatic systems.

### 131 Using advanced speciation techniques to determine the bioavailability of thallium, in rainbow trout (*Oncorhynchus mykiss*) and *Daphnia magna*

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The toxic fraction of waterborne metals is functionally defined as that which passes through a 0.45 µm filter; however, it is understood that this fraction includes metal binding particles which effect bioavailability and toxicity to aquatic biota. Using Asymmetric Flow Field Flow Fractionation (AF4) under ultraclean metal free conditions, the dissolved fraction can be further discriminated, facilitating the identification and quantification of the truly toxic fraction. In this study we analyzed the chemical speciation of thallium (Tl), a trace element associated with the mining of sulfide ores (Pb, Fe, Zn, and Cu). Thallium speciation was assessed concurrently with Tl toxicity to rainbow trout (*Oncorhynchus mykiss*) and the water flea (*Daphnia magna*) under different water chemistries. The mechanism of Tl toxicity was assessed using oxidative stress and ion transport endpoints, and bioaccumulation was related to chemical speciation. Our results suggest that Tl is a metal for which the dissolved ionic fraction is a good proxy for the toxic fraction.

### 132 Accumulation and bio-reactivity of copper and silver complexes in the rainbow trout gut cell line (RTgutGC)

*M. Ibrahim, M. Minghetti, Oklahoma State University / Integrative Biology*

Metal toxicity in aquatic organisms is greatly affected by the chemistry of the water in which they are dissolved. Processes like complexation of metals with inorganic and organic ligands may affect metal toxicity, accumulation, and biological responses (or bio-reactivity). In general, it is believed that only the free ionic forms of metals are bioavailable and metal complexes are not. However, recent studies have suggested that metal complexes may become bioavailable and elicit toxicity. Thus, bioavailability of metal complexes need to be investigated further. Chloride is an important inorganic ligand that forms complexation with metals. Due to its anionic nature, changes in chloride concentration might influence the speciation of metals and consequently their toxicity, accumulation, and bio-reactivity in organisms. In this study, we have tested this question by preparing different synthetic media of varying chloride concentration (0- 146 mM $Cl^-$). Metal speciation of each media was conducted using Visual MINTEQ, a chemical equilibrium software. Ionic $Ag^+$ was dominant in medium with 0 mM $Cl^-$, neutral complex (AgCl) dominated in 1-10 mM $Cl^-$ media, and negatively charged Ag complexes (mainly $AgCl_2^-$) were dominant from 10-146 mM $Cl^-$ media. Copper speciation remained stable across the chloride concentration gradients, mainly complexing to hydrogen phosphate(~42%) or remained in ionic form, $Cu^{2+}$ (~43%). Using the Rainbow trout gut cell line (RTgutGC) as a model, cytotoxicity of $AgNO_3$ and $CuSO_4$ dissolved in the different synthetic media were conducted using two dyes: alamarBlue (indicator of metabolic activity) and CFDA-AM (indicator of cell membrane integrity). In cells exposed to copper, metabolic activity decreased significantly at higher chloride concentrations. Ag toxicity resulted to be more tolerant

at intermediate concentrations (1 to 10 mM) and more toxic in $Cl^-$ free medium and high $Cl^-$ media 25-75 mM $Cl^-$. To investigate metal accumulation and bio-reactivity (metallothinein mRNA levels), cells will be exposed to 500 nM (below NOEC) and LOEC concentrations of each metal dissolved in respective media. Accumulation and bio-reactivity of copper and silver will be determined by ICP-MS and by measuring metallothionein mRNA levels, respectively. This study will shed light on understanding the bioavailability of metal complexes, especially for Ag complexes.

**133 Metal Bioaccumulation and Biomarker Responses in Oysters Exposed to Contaminated Sediments and Hypoxia**

*B. Khan, National Research Council of the National Academies / USEPA Atlantic Ecology Division; A.H. Ringwood, UNC-Charlotte / Department of Biology*

Heavy metal pollution along with fluctuations in dissolved oxygen (DO), pH, salinity, and temperature are major challenges faced by coastal and estuarine organisms. While hotspots of high metal concentrations are limited, many coastal areas have low to moderate levels of contaminants. Many of these areas are also characterized by low DO (hypoxia), which are expanding worldwide due to global warming. Both hypoxia and metals can induce oxidative stress in aquatic organisms via cellular production of reactive oxygen species, and interactions between them can exacerbate oxidative stress. Metal ions can exist in the environment complexed with organic or inorganic matter, and their bioavailability can be affected by water quality parameters such as DO and pH. The overall goal of these studies was to evaluate the effects of hypoxia on metal uptake (Cu, Zn, Mn, Fe) and subsequent toxicity in tissues of Eastern oysters, *Crassostrea virginica*. Oysters are valuable bioindicators of habitat health and play critical roles in maintaining ecosystem integrity. Oyster tissue metal concentrations (gills and hepatopancreas) were measured after four- and eight-day exposures to moderately contaminated sediments under different hypoxic conditions to assess metal uptake. Cellular biomarker responses of oxidative stress were also evaluated (lipid peroxidation to evaluate tissue damage, and total glutathione concentrations for antioxidant status). Increases in gill metal concentrations were found to be related to DO and pH of overlying water indicating that environmental hypoxia can potentially affect metal bioavailability; and oxidative stress was observed. While there were significant correlations between tissue metal levels and biomarker responses, tissue-specific differences were observed. Glutathione levels in gills decreased with increasing Cu and Zn burdens, but increased in hepatopancreas. These studies demonstrate the bioaccumulation of metals from moderately contaminated sediments and the potential effects of hypoxic conditions on bioavailability, as well as the relationships between tissue metal concentrations and cellular biomarker responses of oxidative stress. These studies also demonstrate the value of cellular biomarkers for identifying potential risks of the complex interactions between sediment metals and other environmental parameters that can affect bioavailability and organismal health, and ecosystem sustainability and resilience.

**134 The Influence of Acidification and Copper Exposure on Copper Accumulation and Physiological Responses in the Pond Snail, *Lymnaea stagnalis***

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Atmospheric carbon dioxide ($CO_2$) is rising at an accelerated rated due to various anthropogenic activities. Ocean acidification has recently received much attention; however, effects of $CO_2$ in freshwater environments are less understood. Land-based sources of pollution, such as metals, have also been a noted problem; however, little research has addressed combined exposure of both pollutants to sensitive, calcifying organisms. This study examined copper accumulation and activity of anti-oxidant enzymes, catalase and glutathione peroxidase, in the freshwater common pond snail, *Lymnaea stagnalis,*after exposure to increased $CO_2$ and copper. Results from this experiment demonstrated increased copper accumulation in snails exposed to copper; however, exposure to increased $CO_2$ did not increase the magnitude of copper accumulation. Increased glutathione peroxidase activity was observed as a consequence of $CO_2$ and copper exposure individually, with a greater response in $CO_2$-exposed snails. This study provides insight into mechanisms of toxicity from exposure to multiple contaminants in *L. stagnalis.*

**135 Feeding strategies of stream invertebrates: Linking resource selectivity to metal exposure and uptake**

*T.M. Short, US Geological Survey; M. Hornberger, US Geological Survey / Water Resources Division; R. Keating, US Geological Survey*

Previous studies have shown that life-history strategies are strong determinants of contaminant exposure in stream invertebrates, and that trophic status and feeding behavior are key factors affecting exposure risk. In this study, we examined the importance of resource subsidies (periphyton and seston) as metal sources to invertebrate consumers in a mining-impacted river. Metal concentrations in periphyton and suspended particulates were compared to spatial patterns of metal bioaccumulation in invertebrate primary and secondary consumers. Periphyton was highly efficient in assimilating aqueous metals with uptake rates greatest during early stages of growth and biomass accrual. Examination of invertebrate metal bioaccumulation in relation to feeding trait affinities showed that metal uptake increased markedly in taxa that included periphyton as part of their diet. Similarly, metal concentrations in seston were highly correlated with tissue concentrations in filter-feeding invertebrates ($r^2$=0.74) where filtering comprised >40% of a taxon's food acquisition strategy. Metal bioaccumulation in invertebrates was largely independent of seston transport rates and organic-inorganic composition. Results of gut content analyses will be used to further identify source contributions to metal exposure, and to refine a biodynamic model describing metal uptake and loss kinetics in metal enriched environments.

**136 Uranium uptake in aquatic insect larvae and its loss through insect metamorphosis**

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In 2012, the Department of Interior issued a twenty-year moratorium on uranium (U) mining in federal lands of the Grand Canyon Region, allowing for study of the impacts that this activity has on water and wildlife resources. The Colorado River watershed contains several tributaries that receive U inputs naturally from the land that they drain, and understanding the effects of U on aquatic biota is crucial to understanding the risk of potentially increased U introduction through mining. Aquatic insects create a vital link between aquatic and terrestrial ecosystems. They can carry their contaminant history with them as they metamorphose to become adults, transferring exposure risks to terrestrial consumers, but this varies widely by contaminant. To better understand accumulation and transfer of U to across metamorphosis, we exposed a laboratory mayfly species, *Neocloeon triangulifer,* to aqueous and dietary U as late-stage larvae through metamorphosis. We collected larvae, pre-emergent nymph (PEN), subimago, and imago staged mayflies and measured whole body U content using inductively coupled mass spectrometry (ICPMS). We also used laser ablation ICPMS (LA-ICPMS) to create heatmaps indicating areas where mayflies store U during each stage. Greater than 90% of the U accumulated in the larval stage was lost by the final adult stage; the bulk of that loss (75%) occurred between the larval and PEN stages while the mayflies are fully aquatic. LA-ICPMS qualitatively indicated slight changes in the storage of U from stage to stage within mayflies. The LA-ICPMS showed a loss of U between imago (egg-bearing) and post-partum imago stages, suggesting an additional loss of U through maternal transfer. These findings mirror results from a wide-ranging field collection of larval and adult blackflies (Simuliidae) in the Colorado River and its tributaries within the Grand Canyon. Mean U concentrations in blackfly larvae ranged over an order of magnitude (0.5 – 5.7 µg/g) among sites, but U in adult blackflies at these sites was typically at or below the method detection limit of 0.03 µg/g at these same sites. These results indicate that aquatic insects are not major vectors of U transport from aquatic to terrestrial environments of the Colorado River, and that the risk of U exposure to terrestrial insectivores is low.

## Monitoring, Remediation and Ecotoxicological Assessment of Emerging Contaminants in Soil and Water

**137 Comparing the chemical fingerprints and predicted estrogenicity of conventional soil amendments to sewage sludge**

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Climate change has affected agricultural practices in both the developed and developing world as farmers and landowners search for more drought-resistant and sustainable practices. Soil amendments like composts and animal manures have been used for decades to replace vital plant nutrients, increase water-holding capacity, and improve soil structure and productivity. Simultaneously, wastewater treatment plants are producing millions of tons of sludges annually, most of which remain vastly underutilized in agriculture yet have the potential to serve as a nutrient-rich amendment. This work compares organic contaminants present in California sewage sludge that has undergone various treatment intensities with conventionally used soil amendments (green waste compost, food waste compost, organic dairy manure and non-organic dairy manure) to better understand the risk associated with agriculture-based land application of sewage sludge. Quantitative LC-QTOF-ESI-MS analysis showed that increasing the robustness of sludge treatment is successful at eliminating some contaminants while concentrating others. Suspect screening identified many common pesticides and pharmaceuticals present throughout all amendments. Non-targeted analysis identified hundreds of unique chemical features dividing the sludge-based samples and the conventional amendments. We coupled an estrogenicity-prediction model (VEGA toxicity prediction) to tentatively assigned non-target features to narrow the chemical identification focus to biologically relevant features. Combining quantitative, suspect and non-target analysis with estrogenicity data provides a comprehensive chemical snapshot of the calculated risk associated with land application of sewage sludge on agricultural systems.

**138 Evaluating Native and Naturalized Plant Species for the Phytoextraction of DDT and Dieldrin at Point Pelee National Park**

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Dichlorodiphenyltrichloroethane (DDT) and dieldrin are organochlorine pesticides that were used in Canada until the 1990's. Both pesticides were sprayed in large quantities and have persisted within soils, water bodies and sediments for decades. Previous agricultural practices have resulted in levels of DDT and dieldrin that exceed Canadian soil quality guidelines at Point Pelee National Park (PPNP). Park staff are seeking remediation strategies to remove these contaminants in a cost-efficient manner, while reducing environmental impact and promoting sustainable practices. Phytoremediation is an environmentally feasible technique that uses natural plant processes to remove contaminants from soil. Previous work identified two areas of PPNP with particularly high organochlorine pesticides contamination, which were then selected as test plots for this study. Nine native and naturalized plant species were evaluated for their effectiveness at extracting organochlorine pesticides from contaminated soils via the process of phytoextraction. Microwave extraction was used to quantify levels of DDT and dieldrin in these species. Preliminary results show that two species, *Andropogon geradii* (big bluestem) and *Bromus inermis* (smooth brome), were the most successful at phytoextracting DDT and dieldrin from the contaminated test plots. Phytoextraction using various native species appears to be a viable technology for remediating contaminated sites at PPNP.

**139 Study the Relationship between Physicochemical Parameters of Emerging Organic Contaminants and Plant Uptake in the Presence of Sorbent Materials.**

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The demand of freshwater for irrigation is increasing to produce more food for the global human population. In this context, wastewater (WW) irrigation is a common practice in many countries. However, the presence of contaminants such as hormones, pharmaceuticals, and personal care products (PPCPs) in WW may represent a major risk ad these contaminants may be harmful to the environment and human health. In a field lysimeter study (sandy soil), the uptake of hormones [including estrone (E1), 17β-estradiol (E2) Estriol (E3), Ethinylestradiol (EE2) and progesterone], and PPCPs [ DEET, triclosan, diclofenac, ibuprofen, caffeine, and carbamazepine] was studied in potato plants exposed to contaminated WW. The accumulation was studied in different tissues, including stems, leaves, roots, and tubers. compost and biochar were added in specific lysimeter treatments and the translocation of contaminants to potatoes was investigated. In total, six treatments (control, compost, biochar 1%, biochar 3%, biochar 1% + compost, and biochar 3% + compost,) were tested in triplicate. The results are discussed in the context of the physicochemical parameters (such as water solubility, octanol-water partition coefficient, and acid dissociation constants) of organic contaminants.

**140 Selected polychlorinated biphenyls (PCBs) congeners and dichlorodiphenyltrichloroethane (DDT) in fresh root and leafy vegetables**

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Persistent organic pollutants (POPs) are dangerous and toxic pollutants reckon to cause adverse effects on human and animal health, including death. POPs such as polychlorinated biphenyls (PCBs) and pesticides are subtly released into the environment from industrial and agricultural use. Global circulation is due to their trans-boundary transport capacity, contingent on aerodynamic and hydrological properties. Plants have capacity to take-up POPs, and these bio-magnify along heterotrophic transfer pathways. In this study, levels of selected 6-PCB congeners and 3- DDTs in some leaf and root vegetables were investigated. Leaf and root vegetables were collected from different horticultural farms areas in Cape Town. The 6-PCBs and 3-DDTs were recovered from the samples using solid phase extraction(SPE), followed by GC-MS analysis. The ∑PCBs and ∑DDT (on-whole basis), were ranged: 90.93-233.87 µg/kg and 38.92-66.14 µg/kg respectively. The 3-PCBs and 6-DDTs levels were slightly higher in leaf vegetables compared to root vegetables. The detection of PCBs and DDTs in the vegetables suggest the probable use of PCBs containing pesticides. Although the observed concentrations were below the WHO maximum residue limits, consumption of such contaminated leaf and root vegetables portend a health risk.

**141 Remediation of polycyclic aromatic hydrocarbons (PAHs)-contaminated soil using activated carbon supported bimetallic particles-activated persulfate**

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With the development and growth of several industrial activities, polycyclic aromatic hydrocarbons (PAHs) have appeared and accumulated in soils and groundwater. Recent years, PAHs have become one of the major contaminants in the world because of their typical features of low solubility, eco-toxicity, mutative and carcinogenic to human. Chemical oxidation is a promising technology for the remediation of soils contaminated with PAHs. In this study bimetallic materials supported on activated carbon (AC) were synthetized to activate persulfate for the degradation of 19 kinds of PAHs, such as Naphthalene (NAP),2-Methylnaphthalene (2-MET), Acenaphthylene (ANY), Fluorene (FLU), Phenanthrene (PHE), Anthracene (ANT), Fluoranthene (FLT), Pyrene (PYR), Benzo(a) anthracene (BaA), Chrysene (CHR), Perylene (PER), Benzo(b)fluoranthene (BbF), Benzo(k)fluoranthene (BkF), Benzo(e)pyrene (BeP), Benzo(a) pyrene (BaP), Indeno(1,2,3-cd)pyrene (IPY), Dibenzo(a,h)anthracene (DBA), Benzo(g,h,i)perylene (BPE). Five kinds of bimetallic catalysts, including AC-supported metal such as Fe, Fe/Cu, Fe/Ni, Fe/Pd and Fe/Pt were synthesized by liquid phase reduction method. The characterization by Brunauer-Emmett-Teller (BET) and X-ray diffraction (XRD) showed that the prepared composites have larger specific surface area, higher intensity and high loaded on AC of bimetallic materials. Temperature, reaction time were studied to observe the removal efficiency of 19 kinds of PAHs from soil-water system. Higher PAHs removal efficiencies were achieved when Fe-AC was used as activator, the removal efficiency of NAP, PER orBbF was about 60%~70%, while other 16 kinds of PAHs showed higher resistance to oxidation, achieving 80%~100% removal after 72 h combined with heating (50?). When use AC-Fe/Ni as activator, 17 kinds of PAHs was completely removed except PER orBbF. Similar results were obtained when activation was AC-Fe/Cu at the same reaction condition. While persulfate was activated by AC-Fe/Pd or AC-Fe/Pt, the removal efficiency of 16 kinds of PAHs showed slightly lower than activators AC-Fe/Ni and AC-Fe/Cu, except that NAP, PER or BbF was near 70%. AC-supportedbimetallic particles-activated persulfate combined with heating process showed higher removal efficiency than persulfateoxidation processes in the remediation of PAHs-contaminated soil. Taking into account the high removal efficiency and the acceptable remediation times the technology is suitable for its aimed purpose.

**142 Survey of Brominated Flame Retardants (PBDE and BB 153) in the Diep and Kuils Rivers, Cape Town, South Africa**

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Brominated flame retardants (BFRs) are substances used in plastics textiles, electronic circuitry and other materials to prevent fire. Their presence in the environment are generating increasing concern because of their potential human and ecological health risks. They belong to the group of emerging contaminants and are classified endocrine disruptors. Studies on them are still scanty in South Africa. This study reported the concentrations of PBDE and BB153 congeners in waters and bottom sediments of two rivers in Cape Town, South Africa. There analyses and structural elucidation were done by GC-ECD and GC-TOF-MS, respectively. The results indicated concentrations of BPDEs in the range of 0.06 – 2.47 ng/g in bottom sediments of Diep River while levels were relatively higher in the sediment of Kuils River. Mean concentrations of BB 153 in the Diep River were in the range of ND – 0.39 ng/g while it varied between ND – 1.21 ng/g in the Kuils River. Effluent discharges from nearby wastewater treatment plants were identified as important sources of the PBDEs in the rivers while grey water intrusion could contribute to upstream contamination. The work confirmed the contamination of the water systems with BFRs with potential for ecological and human health effects, especially if the water from the rivers were used for domestic purposes without treatment and the EDCs were not removed or if the waters were used for irrigation purposes in agriculture.

**143 Pollution Characteristics and Ecological Risk Assessment of PFASs in Jiulong River Estuary-Xiamen Bay, China**

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Per- and polyfluoroalkyl compounds (PFASs) are a new type of persistent organic pollutants (POPs) closely concerned by the world. They were detected in various media from many countries include China, one of the major producers of PFASs. Jiu-long river estuary-Xiamen Bay is an important water source in the Xiamen area, China. This paper studied the pollution characteristics and ecological risk assessment of PFASs on Jiulong river estuary-Xiamen bay. The concentrations range of 28 kinds of PFASs (including 13 perfluorinated carboxylic acids (PFCAs), 5 perfluorinated sulfonic acid (PFSAs), and 10 precursors), was 0.34-100.33 ng L-1 in the water with a significant seasonal variation. The PFASs of the surface water in the dry season (12.94-100.33 ng L-1) was much higher than those (0.41-5.88 ng L-1) in the wet season. PFOS was the main medium-chain PFASs, PFBS was the main pollutant of PFSAs, and PFBA, PFPeA, PFHxA were dominant in PFCAs. The PFASs in the sediments of the Jiulong river estuary – Xiamen bay was 4.64-7.42 ng g-1 dw. PFOS was the main pollutant. The adsorption ability of sediment increased as the carbon chain and salinity increased according to the sediment-water partition coefficient. The ecological impact of PFASs was also assessed using AIST-MeRAM model. The results showed that PFASs constituted no ecological threats to the research area so far, measured by the Margin of Exposure (MOE) indicator and Species Sensitivity Distribution method (SSD).

**144 Quantification and probabilistic risk assessment of selected β-lactam antibiotics in the Diep River water samples**

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Pharmaceuticals are widely used in human and veterinary residues. Residues of pharmaceutical compounds have been reported to be present in biological samples and environmental matrices. Occurrence of pharmaceuticals has been linked to many global health and ecological issues. These include antibiotic resistance and endocrine disruption among others. In this study, a method was developed for the measurement of three antibiotics in water samples. The method was used for qualitative and quantitative determination of the three β-lactam antibiotics (amoxicillin, ampicillin and chloramphenicol) in surface water samples collected from the Diep River. Levels of the compounds measured were subjected to probabilistic risk assessment to predict the possibility of exceeding regulatory values. Mean concentrations of quadruplicate samples of water collected in winter and spring months from five sampling points were ranked using Weibull's probabilistic approach and centile rankings. This was employed to examine the likelihood of exceedance of predicted no effect concentrations set by international regulatory agencies. The three β-lactam antibiotics-amoxicillin (AMX), ampicillin (AMP) and chloramphenicol (CHLR) occurred at variable concentrations in the Diep River. The levels detected ranged between < DL – 13.29 µg/L and < DL – 11.55 µg/L in winter and spring respectively for AMX. Corresponding values for AMP were < DL to 1.24 µg/L and < DL – 5.27 µg/L in CHLR was not detected in all water samples analyzed. Results of probabilistic risk assessment of the compounds will be presented.

## Exposure Assessment and Modeling for Ecological Risk Assessment of Veterinary Pharmaceuticals and Pesticides

**145 In vitro Assessment of Pyrethroid Bioaccessibility via Particle Ingestion**

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Due to their intensive use in agricultural and residential pest control, human exposure to residues of multiple pyrethroids frequently occurs. Pyrethroids have exceptionally high affinity for solid particles, highlighting the need to understand human exposure through oral ingestion of contaminated soil or dust particles. In this study, we used artificial gastrointestinal fluids to measure the desorption or bioaccessibility of eight current-use pyrethroids in soil and dust samples. Tenax was further included as a sink in parallel treatments to simulate the effect of removal due to transfer of pyrethroids to lipid membranes. The use of 0.4 g of Tenax in 20 mL digestive fluids resulted in rapid and efficient trapping of pyrethroids, and further, greatly enhanced bioaccessibility. In the artificial digestive fluids without Tenax, 6.0-48.0% of pyrethroids was desorbed over 21 h, and the fractions increased by 1.6-4.1 folds to 21.5-79.3% with the Tenax sink. Therefore, 6.0-79.3% of soil or dust-borne pyrethroids may be considered bioavailable upon ingestion. While protein and sucrose increased the estimated bioaccessibility, co-presence of lipid (vegetable oil) decreased the bioaccessibility of pyrethroids, likely due to competitive phase partition. Pyrethroids were also found to be unstable in the artificial intestinal fluid containing pancreatin, further decreasing the potential bioaccessibility of pyrethroids on soil or dust particles. The limited bioaccessibility should be considered to refine the prediction of human exposure and risk through oral ingestion of pyrethroid residues.

**146 The Distribution and Concentration of Antibiotics in Urban Streams, Rural Streams, and Rural Groundwater in the Piedmont of North Carolina**

*A.D. Gray, University of North Carolina at Greensboro / Biology; D. Todd, University of North Carolina at Greensboro / Chemistry; A.E. Hershey, University of North Carolina at Greensboro / Department of Biology*

Classification of pharmaceuticals as "contaminants of emerging concern" has led to numerous studies investigating the ecological impacts of these compounds. Antibiotics are the most widely used pharmaceutical product on the market, leading to much attention due to their role in developing resistant bacteria. Antibiotic pollution in streams is of importance because streams play a crucial role in the transport of water from inland areas to coastal waters as well as to rivers and lakes. The presence of antibiotics in streams demonstrates the capacity for antibiotics to be transferred from inland areas into larger water bodies. Antibiotics entering streams can arise from various sources. In urban areas, antibiotics of human and veterinary origin can enter streams due to runoff or leaching from surrounding areas, but most notably from wastewater discharge that releases effluent directly into streams. In rural areas, antibiotics can enter streams from antibiotic application in the maintenance of livestock, which through runoff and leaching, can contribute to veterinary antibiotics being present in rural streams and groundwater. The interaction between groundwater and surface water provides a mechanism for contaminated water to not just have ecological implications, but also human health implications because groundwater is often the source of rural drinking water. The present study investigated 16 residential rural well sites in Guilford, Alamance, and Randolph County, North Carolina. Along with these 16 well sites we also investigated 16 stream sites in close proximity to these residential properties, and 6 urban streams sites, 3 of below WWTFs and 3 above WWTFs. Mass spectroscopy work with the LTQ Orbitrap instrument detected 13 antibiotics in these compartments over 3 different seasons (fall, winter, and spring). These detected antibiotics have applications that originate from both human and veterinary uses. Results indicate that land use and population are key factors in the distribution of antibiotics in stream ecosystems and that groundwater contamination is of concern due to the role it plays in feeding into stream surface waters and rural drinking wells.

**147 Comparison of OECD 308 and OECD 309 Simulation Studies Investigating the Fate of Human Pharmaceuticals in Water-Sediment Systems**

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Simulation studies are fundamental in determining the potential for persistence and for the assessment of risk of exposure. The OECD 308 and 309 Guidelines describe simulation studies designed to assess the biotransformation of distribution behavior in water-sediment systems. Since the European Medicines Agency (EMA) environmental risk assessment (ERA) testing guideline was finalized in June 2006, the OECD 308 Water-Sediment transformation test has been routinely conducted in Phase II Tier A testing for all human pharmaceutical marketing authorization applications. The 308 test design, a stagnant and stratified sediment layer:pelagic river water layer, does not represent a typical wastewater treatment facility discharge into a river, but rather that of an irrigation ditch; a scenario which is considered more applicable to pesticide application. Results from the 308 include half-life values in water, sediment and total system; amount of compound shifting to sediments, and non-extractable residue (NER) formation; identity of transformation products (if >10%); amount of $CO_2$ formation; and total mass balance and distribution in the test system. The OECD 309 "Aerobic Mineralization in Surface Water" guideline defines a simulation study representative of discharge into a moving water system, with a fully aerobic mixed water-suspended sediment test design. Similarly, results from the 309 include total system half-lives; NER formation; identity of transformation products (if >10%); and amount of $CO_2$ formation. For the past several years, Pfizer's strategy has been to include OECD 309 as part of the ERA testing package in order to fully appreciate the potential fate of APIs discharged into natural receiving waters. The aim of this report is to relate the data obtained from OECD 308 and 309 studies for several pharmaceuticals, systematically comparing how they behave in both 308 and 309 water–sediment systems by exploring relationships between biotransformation rate constants, NER formation, and mineralization across the test systems. The goal of this study is to determine if the outcomes from the 28 day 309 study can be used to predict the potential for similar indications of persistence or exposure risk obtained in the 100 day 308 study.

**148 Loading of Pesticides to Wastewater Catchment from Flea and Tick Topical Products**

*J. Teerlink, California Department of Pesticide Regulation / Surface Water Protection Program; Y. Xie, R. Budd, California Department of Pesticide Regulation; T.M. Young, University of California-Davis / Civil and Environmental Engineering*

Flea and tick treatments used on domestic animals contain pesticide active ingredients that serve as a source to wastewater treatment catchments. Pesticides can enter wastewater directly through routine bathing or indirectly through cleaning surfaces the pet has come in contact with such as companion humans, pet bedding, and other residential surfaces. The California Department of Pesticide Regulation (DPR) is working to evaluate the relative contribution of currently-registered flea and tick treatments to total pesticide loading in wastewater catchments and improve registration evaluation and post-use risk assessment modeling capabilities for pet products. DPR conducted a dog-wash study to quantify the fraction of fipronil in spot-on products that washed off during routine bathing of volunteer dogs. Dogs were washed 2, 7, or 28 days post application with an average of 21 ± 22, 16 ± 13, and 4 ± 5 % wash-off respectively. There was measurable fipronil in all samples with a total mass during a single bathing event ranging from 3.6–230.6 mg per dog. A subsequent study measured effluent from a pet grooming operation at the discharge point to a wastewater system and found pesticide active ingredients used in common topical treatments (imidacloprid, permethrin, bifenthrin, cyfluthrin).

The same set of samples was analyzed using nontarget suspect screening by LC-Q/TOF methods. The presence of additional compounds found in ingested products and topically-applied pet treatments were tentatively identified with a high degree of confidence (i.e., not confirmed with a standard). An improved understanding of direct and indirect down-the-drain transport of pet products is essential to develop pre-registration and post-use environmental risk models.

**149 Identification of Herbicide Source Areas and Dominating Transport Processes in a High Agricultural Intensity Catchment**

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The occurrence of herbicides in surface waters of intensively cultivated catchments can originate from a variety of sources. These include transport via runoff and erosion during storm events, subsurface transport through lateral flow and through subsurface tile drainages, and from spray drift during applications. The Soil and Water Assessment Tool (SWAT) is widely used in the United States and the EU for catchment scale hydrologic and water quality modeling of non-point source chemicals in the environment. The SWAT model was applied to a 992 ha agricultural catchment in the Flanders region of Belgium to help in better understanding the sources of the herbicide detections observed in daily sampling over 3.5 years at two locations along the catchment's primary stream. The SWAT model was calibrated to observed flow and chemical monitoring data, then used to characterize the relative contributions of herbicides via surface processes, subsurface processes, and spray drift. In addition, very vulnerable fields with significant contributions to surface water exposure were identified. A quantitative comparison between monitoring data and simulated exposure profiles was made to single out those high residue concentrations that could not be attributed to any of these traditionally considered exposure pathways, and could ultimately be only explained by point source contributions. The model results demonstrate that SWAT is capable of simulating streamflow in a small agricultural catchment, and is capable of simulating diffuse source pesticide concentrations. This allowed application of an approach that incorporated model uncertainty analysis in distinguishing between diffuse source dominated high concentrations from those most likely affected by point sources. The SWAT model also proved useful in identifying the spatial variability in the dominant transport processes contributing pesticide residues to the stream. While surface runoff of soluble pesticide was the major non-point source contributor on most fields, lateral subsurface flow was found to be important as well, especially in the western portion of the catchment. Spray drift is likely the least significant contributor at the catchment scale. Overall, the analysis of monitoring data and modeling results shows that the potential for reducing herbicide concentrations in the study catchment can be addressed by mitigating both point source contributions from farmyards as well as diffuse sources.

**150 Modeling environmental exposure to Revalor-XR from beef feedlots and manure-amended crop fields**

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Revalor-XR is an extended-release veterinary drug for implant in steers and heifers that contains trenbolone acetate and 17β-estradiol (17β-E2) as active pharmaceutical ingredients (APIs). As part of the Environmental Assessment (EA) for approval of this drug in the US, a novel exposure assessment was conducted. The focus was on characterizing exposure of surface water due to runoff and leaching from beef feedlots and manure-amended fields, both for generic farm scale and watershed scale for selected watersheds with high-density beef feedlots and manure-amended soils. Both APIs are metabolized in situ resulting in the excretion of 17β-trenbolone (17β-TB), 17α-trenbolone (17α-TB), trendione (TBO) and 17β-E2, 17α-estradiol (17α-E2), and estrone (E1). The relevant environmental fate data for each of the individual compounds determined in laboratory studies were aggregated to generate representative values for the surrogate trenbolone compounds and estradiol compounds. The runoff potential from the feedlots was calculated using the USDA-NRCS' approach accounting for water balance (TR-55 method) and mass balance of the surrogate compounds. The runoff potential from the manure-amended crop fields was evaluated using the USEPA's EXPRESS model. The potential for leaching, both from feedlots and crop fields was evaluated using the USEPA's SCIGROW model. Finally, surface water exposure for selected watersheds was evaluated using USEPA's BASINS model. The generic farm scale exposure assessment demonstrated that the major pathways of exposure were runoff from either feedlots or manure amended crop fields, with leaching pathways being only minor contributors. The exposure assessment of the surrogate compounds on the watershed scale was thus performed by considering the runoff pathways only. The exposure assessments further showed that the concentrations of the surrogate compounds in surface water varied with the generic farm scale scenarios resulting in higher concentrations (runoff from feedlot > runoff manure-amended crop field) compared to the watershed scale scenarios. The assessment at the watershed scale demonstrated that it is highly unlikely that the compounds associated with Revalor-XR would have any significant environmental impacts when used according to the Revalor-XR label. The environmental assessment supported a Finding of No Significant Impact by the U.S. Food and Drug Administration from the use of Revalor-XR in beef steers and heifers in the US.

**151 Model the effectiveness of vegetated filter strips in reducing contaminants in feedlot runoff**

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The National Pollutant Discharge Elimination System (NPDES) regulations require concentrated animal feeding operations (CAFO) with greater than 1000 head beef cattle to contain feedlot runoff in settling basins designed to hold runoff from a 25-year 24-hour rainfall. CAFO with less than 1000 head may discharge feedlot runoff to nearby waters under NDPES permits if there are certain best management practices (BMPs) in place like settling basins or vegetated filter strips. Runoff from feedlots may contain nutrients or veterinary pharmaceutical residues excreted in animal waste, which under some circumstances could be potentially harmful to aquatic organisms if released directly to nearby surface waters. The objective of this presentation is to model the effectiveness of vegetated filter strips (VFS) in reducing contaminants in feedlot runoff using the WINPRZM and VFSMOD models. Effectiveness of VFS in reducing nutrient concentrations in feedlot runoff will be presented as a case study. WINPRZM was enhanced to simulate runoff from an earthen or concrete uncovered beef feedlot. The model predicts the daily edge-of-field mass of nutrients and other constituents in runoff generated on a feedlot due to precipitation. The feedlot algorithm can model daily manure accumulation, various chemical administration patterns, and the periodic scraping of feedlots. The model uses the SCS curve number method to estimate runoff, a non-uniform mixing model to extract constituents from manure, and the manure erosion equation from the APEX model. The daily edge-of-field mass loadings estimated by WINPRZM are then input to the VFSMOD model which estimates the reduction of loadings based on the size of VFS and the resulting concentrations in runoff discharging from the VFS.

**152 The influence of downwind vegetation and distance from application on spray drift deposition to non-target plants**

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This study is the first in a two-part study designed to determine the distance downwind from treated areas at which non-target plants are no longer significantly affected by applications of an herbicide formulation. The purpose of the initial study was to measure spray drift deposition following application of a "blank" formulation (i.e., no active ingredient) that contained a fluorescent tracer dye for two scenarios: spray drift collectors located downwind on bare ground (worst-case exposure) and collectors located downwind in edge vegetation (typical exposure). The study was conducted from 17-20 October 2017 at an agricultural site in Stilwell, KS in Johnson County using a ground boom sprayer and coarse spray nozzles. Deposition collectors (horizontal and vertical) and plants (navy bean and lettuce) were placed along three transect lines at locations 5, 10, 20, 50, and 100 ft downwind from the edge of the application area. For each exposure scenario, there were four spray swaths that ran perpendicular to the off-field transects. Deposition was always < 1% of applied formulation at 5 feet downwind of the application area and < 0.1% at 50 feet downwind. In addition, the in-vegetation deposition was lower than the bare ground deposition for the more vertical collectors (nylon string, navy bean). However, bare ground and in vegetation deposition were similar for the more horizontal collectors (Mylar card, lettuce). It appears that the presence of vegetation partially blocked lateral transport of the formulation but did not block deposition from above. The second phase of this study is scheduled for October, 2018 and will involve the use of the formulated active ingredient to determine a downwind distance at which the herbicide drift does not significantly affect vegetative growth of lettuce and navy bean placed on bare ground and in edge vegetation.

## Advances in the Toxicological Assessment of PAHs

**153 The use of mass spectrometry to track sources of oil (PAHs) contamination in the marine environment**

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Polycyclic aromatic hydrocarbons (PAHs) are well known contaminants, ubiquitously present in the habitat and spawning areas for Atlantic cod (*Gadus morhua*). The Atlantic cod is a key species and a globally important food source, thus continuous monitoring of PAHs is considered highly valuable to ensure ecosystem sustainability and human food safety. PAH adducts to plasma proteins are used as sensitive biomarkers of PAH exposure in humans and other species, thus the presence of PAH protein adducts in Atlantic cod plasma was investigated to identify PAH protein adduct biomarker candidates of exposure to PAHs. Blood plasma samples were collected from Atlantic cod exposed to single PAHs (i.e. naphthalene, chrysene and their corresponding dihydrodiol metabolites, (-)-(1*R*,2*R*)-1,2-dihydronaphthalene-1,2-diol and (-)-(1*R*,2*R*)-1,2-dihydrochrysene-1,2-diol) and to crude oil. Samples were analyzed by shotgun tandem mass spectrometry (MS) and the resulting MS data were evaluated to screen for proteins susceptible to adduct formation with naphthalene and chrysene and other PAH compounds. Furthermore, a wildcard modification search was performed to obtain additional information regarding potential modifications other than the targeted metabolites. Amino acid adductation sites and metabolites involved in PAH adductation will be presented. Forty-four proteins were found to bind PAHs. The proteins reported with the highest numbers of PAH adducts were alpha-2-macroglobulin-like proteins, apolipoproteins B-100-like proteins and an alpha-2-HS-glycoprotein, which may be the first targets for PAH protein adduct biomarker development in future studies. Furthermore, the wildcard results revealed two highly abundant protein modifications on a hemopexin and alpha-2-macroglobulin protein, and their presence may be of importance for future research. This work provides also the first insight into PAH protein adducts of Atlantic cod plasma, generating valuable knowledge for the development of highly sensitive biomarkers of PAH exposure.

**154 Characterization of PAH contamination using dreissenid mussels in the Great Lakes**

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The National Oceanic and Atmospheric Administration, National Centers for Coastal Ocean Science Mussel Watch Program (MWP) conducts basin-wide monitoring and place-based assessments using dreissenid mussels as bioindicators of chemical contamination in the Laurentian Great Lakes. A multivariate statistical analysis of this long-term combined dataset revealed three distinct PAH concentration patterns by site type: offshore, nearshore and river-harbor, and relationships between total PAH concentration in dreissenid mussel tissue, impervious surface percentages, and PAH relative concentration patterns were explored. To characterize bivalve health, metabolomics, DNA damage, and biomarkers were measured at selected sites and were linked to PAH tissue concentrations to better understand PAH exposure and bioeffects.

**155 Effects of Deepwater Horizon crude oil exposure on the developing teleost kidney**

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Much has been learned with respect to the developmental toxicity of crude oil exposure to teleost fish in the years following the Deepwater Horizon oil spill. Among the key findings are several lines of evidence that specific crude oil constituents, namely polycyclic aromatic hydrocarbons (PAHs), impair the function and proper development of the teleost heart. The resulting reduction in cardiac output is believed to elicit a characteristic suite of downstream effects (e.g., pericardial and yolk sac edema) and likely impairs the proper development of other organs, such as the kidney. While there is some evidence that exposure to individual PAHs impairs development of the early stage kidney (i.e., pronephros), little is known regarding the effects on the pronephros following exposure to complex crude oil mixtures at environmentally relevant concentrations. Furthermore, it is unknown whether early life stage (ELS) effects arising from short-term exposures result in long-term or latent effects on kidney function. To address these knowledge gaps, time-course and dose-response exposures to zebrafish embryos were performed using high energy water-accommodated fractions (HEWAFs) of DHW slick oil. Transcriptional changes in genes with various structural, functional and signaling roles specific to different regions of the developing pronephros (e.g., glomerulus, pronephric tubule and pronephric duct) were assessed by QPCR and whole mount in situ hybridizations. Additionally, the long-term effects of ELS crude oil exposure on kidney function and development was examined using histological assessments and labelling assays to assess nephron function in adults. Results demonstrate transcriptional changes in key genes involved in early kidney development and function and that early stage impairment of normal kidney development might translate into long-term impairment of teleost kidney function. This research was made possible by a grant from The Gulf of Mexico Research Initiative.

**156 The effects of oil-exposure on ammonia and urea excretion in mahi-mahi (*Coryphaena hippurus*) early life stages**

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The timing and location of the 2010 *Deepwater Horizon* (DWH) incident within the Gulf of Mexico coincided with the spawning of many commercially and ecologically important fish species, such as mahi-mahi. Early life staged mahi exposed to environmentally-relevant crude oil concentrations have displayed increased oxygen consumption suggesting an increased metabolic rate which is mainly fueled by protein catabolism from the yolk sac. In teleosts, protein is usually catabolized into ammonia or urea. Recent studies by our lab revealed that, similar to other teleosts, mahi avoid the toxic build-up of ammonia by being largely ureotelic during the embryonic stage and gradually switch to being ammoniotelic for less ATP cost at the time of hatch (42-48 hpf). The excretion process happens mostly at the gills, where Rh protein family (Rhag, Rhbg, Rhcg1 and Rhcg2) works as ammonia transporters and UT works as urea transporter. In this study, mahi embryos and larvae were exposed to 2%, 4% and 6 % HEWAF (6.4, 14.2, 20.7 µg $l^{-1}\sum$50 PAH) to investigate the impacts of crude oil on ammonia and urea excretion by examining gene expression changes of related transporters over the initial 102 hpf of life. Crude oil was found to significantly increase mRNA levels of ammonia transporters in embryos until hatch but not urea transporters. Each ammonia transporter's response to crude oil was differently among development which suggests varied function of the transporters. The upregulated mRNA levels of transporters support observations of increased metabolic demand fueled by protein metabolism, especially during hatch when any physiological change could impact larvae survival. Our results contribute to understanding the underlying mechanism of toxicity from crude oil in early life staged pelagic fish. Efforts to measure the ammonia and urea excretion rate after oil-exposure are currently ongoing. This research was made possible by a grant from The Gulf of Mexico Research Initiative. Grant No: SA-1520.

**157 Is the stress response of Gulf toadfish affected during a one-week DWH oil exposure and what is the potential for recovery?**

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The polycyclic aromatic hydrocarbons (PAHs) released in the Deepwater Horizon (*DWH*) oil spill have been found to impair the glucocorticoid stress response of vertebrate species found in the Gulf of Mexico (GoM). Specifically, reduced plasma cortisol levels measured in marine mammals found in areas contaminated by *DWH* oil and in teleosts exposed experimentally suggest a reduced ability to properly respond to natural environmental stressors. The mechanisms for this remain unclear; inhibition in cortisol secretion may be due to pituitary fatigue through over-stimulation of the hypothalamic-pituitary-interrenal (HPI) axis or could be via an inhibition of cortisol biosynthesis through interactions of PAHs and the aryl hydrocarbon receptor (AhR). Gulf toadfish, *Opsanus beta*, a GoM resident, will serve as a model species to understand the effects of *DWH* PAHs on the HPI axis and the potential for recovery. Using a flow-through system, toadfish were continuously exposed to control conditions or three environmentally relevant concentrations of PAHs from *DWH* high energy water accommodated fraction (HEWAF; ∑PAH = 0 – 10 µg?$L^{-1}$) for 0, 4 h, 8 h, 24 h, 3 days, 7 days followed by recovery for 7 days with sampling occurring at the same time periods. At these exposure and recovery time points, corticotropin releasing factor (CRF) mRNA expression in the hypothalamus will be quantified using qPCR to determine if PAHs are perceived as a stressor, thus stimulating the HPI axis. Circulating adrenocorticotropic hormone (ACTH) that is released from the pituitary will be quantified in the blood plasma to determine if oil exposure has the potential for pituitary fatigue. Additionally, mRNA expression of the interrenal melanocortin 2 receptor (MC2R), the steroidogenic enzymes StAR, cytochrome P450 side chain cleavage (P450scc), and 11β-hydroxylase, and plasma cortisol concentrations will be measured to determine if PAHs may have the potential to affect the responsiveness to ACTH and subsequent cortisol production and secretion. Cytochrome P4501A1 (CYP1A) mRNA expression will also be measured throughout HPI axis tissues to measure potential involvement of the AhR. Temporal changes in the components of the HPI axis in response to different PAH concentrations and exposure and recovery lengths will allow us to better understand if PAHs are overstimulating the HPI axis or impairing the HPI axis through the AhR pathway and will serve as a foundation for future chronic exposures.

**158 Associations between microscopic hepatic changes and PAH accumulation in wild-caught Gulf of Mexico Golden tilefish**

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The Center for Integrated Modeling and Analysis of Gulf Ecosystems (C-IMAGE) completed a series of Gulf-wide cruises, 2012-2017, to study the prevalence of fish disease and polycyclic aromatic hydrocarbon (PAH) contamination in Gulf of Mexico demersal fishes. Golden tilefish (*Lopholatilus chamaeleonticeps*) were chosen as a target species due to their strong association with sediments, relatively high levels of a biomarker of PAH exposure (x = 280 ug/g biliary naphthalene metabolites) and accumulation of PAHs in liver tissue (x = 2700 ng/g w/w total PAH). Fish were collected via demersal longlining from stations (n = 23) on the United States and Mexican continental shelf at depths typically 200-450 meters. A variety of tissues were sampled for contaminant analyses and pathology, including liver and bile presented herein. Histological evaluations of 239 livers were performed by a board-certified veterinary pathologist at Fishhead Labs LLC. Biliary PAH metabolites were analyzed semi-quantitatively using standard high performance liquid chromatography with fluorescence detection (HPLC-F) methods. PAHs and alkylated homologs were extracted from liver tissue using QuEChERS Enhanced Matrix Removal (EMR) and quantified via gas chromatography tandem mass spectrometry (GC-MS/MS). Preliminary results from individuals (n = 163) collected in the north-central Gulf around the De Soto Canyon show widespread occurrence of hepatic changes (99% of fish evaluated), with the most frequent being glycogen-type vacuolar change (76%), pigmented macrophage aggregates (61%) and biliary fibrosis and duplication (49%). Occurrence of neoplasms was rare (n=1; hepatocellular adenoma), however, approximately 10% of individuals examined exhibited foci of cellular alteration (FCA), often considered "preneoplastic" lesions. Preliminary analysis suggests associations between increased occurrence of atrophy, FCA and biliary fibrosis with biomarkers of PAH exposure, as well as associations between increased occurrence of glycogen-type vacuolar change, FCA and biliary fibrosis with PAH accumulation in hepatic tissue. Further analysis of spatial and temporal trends in hepatic changes and PAH contamination is underway. This study is the first to evaluate the effects of chronic anthropogenic pollution on Gulf of Mexico Golden tilefish health.

**159 Photo-induced toxicity of polycyclic aromatic hydrocarbons and relationship to body burden in two amphibian species**

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Certain polycyclic aromatic hydrocarbons (PAHs) exhibit photo-induced toxicity with ecologically relevant intensities of ultraviolet (UV) light, resulting in increased mortality and developmental effects in aquatic organisms. Photo-induced toxicity of PAHs is dependent on many factors including internal PAH concentration, which can vary across species and dependent on toxicokinetics. Early life-stages of amphibians may be particularly susceptible to PAH photo-induced toxicity as they can accumulate PAHs and often develop in shallow ponds where they are exposed to UV light. The objective of this research was to assess and compare that

photo-induced toxicity in two amphibian species following exposure to PAHs and determine whether relative differences in sensitivity between species is related to accumulated levels of specific PAHs. *Lithobates sylvaticus* (wood frog) and *Xenopus laevis* (African clawed frog) early life stage tadpoles were exposed to three individual PAHs (benzo(a)pyrene, anthracene, and naphthalene). Tadpoles were allowed to accumulate chemicals over 8 h at which time samples were taken for body burden analysis. Tadpoles were then transferred to clean water under UV exposure for 12 h and then grown out without UV or chemical exposure until the end of the 96 h toxicity test. *Xenopus* and wood frog tadpoles exposed to anthracene or benzo(a)pyrene followed by UV light had higher mortality when compared to either PAH alone. *Xenopus* had decreased length and increased deformities when exposed to UV light alone regardless of chemical treatment. Wood frogs exposed to 2 and 20 ug L-1 anthracene, and 100 ug L-1 benzo(a)pyrene had a significantly higher body burden compared to *Xenopus* exposed to the same concentrations. The LC50 value for *Xenopus* exposed to anthracene and UV light was 5 ug L-1, while the LC50 value for wood frogs exposed to anthracene and UV light was 124 ug L-1 leading us to conclude that for anthracene, toxicity thresholds based on *Xenopus* would be protective for the wood frog. The LC50 values for benzo(a)pyrene and UV light were more comparable between species with 45 ug L-1 for *Xenopus* and 17 ug L-1 for wood frogs. Overall, this research demonstrates that photo-induced toxicity is an important consideration in the hazard assessment of PAHs, provides new data on the acute phototoxic effects of PAHs in two amphibian species, and indicates that species differences in response are not driven by differences in PAH uptake.

**160 Does predation risk increase with crude oil exposure?**

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As a pervasive toxicant, crude oil is a major ecological concern worldwide. The suite of cardiotoxic injuries sustained by oil exposure, primarily driven by three-ring polycyclic aromatic hydrocarbons, has been well described in fish species. This impaired cardiac function has been shown to reduce aerobic scope and swim performance in a number of species. Recently there has been evidence to suggest that fish also suffer neurological and cognitive function impairment following oil exposure. However, little is known about the ecological implications of these sub-lethal injuries, including predation risk. This study investigated the possible impacts of oil exposure on predation risk in small juvenile red drum, *Scianeops ocellatus*. A mesocosm-style approached was used immediately following an acute exposure. We first examined routine habitat utilization behavior after which common predators were introduced and time to mortality was monitored. Using an environmentally realistic oil concentration, swim speed ($U_{crit}$) was used as a marker of impaired physiological performance. Results suggest that while there was no difference in aerobic scope, oil exposed fish had a significantly lower $U_{crit}$ than controls. In the mesocosm-style study, oil exposed groups were more likely to utilize open sandy bottoms than control fish who preferred either seagrass areas or the wall. Consequently, the time to 50% mortality by predation was also significantly lower in oil exposed fish. These findings demonstrate that sub-lethal oil exposure can have important downstream ecological consequences.

## Water: Bringing Science and Engineering Together to Address Impacts of Climate Change on Water Supply and Water Quality

**161 Preparing for Droughts in a Changing Climate**

*E. Hanak, PPIC Water Policy Center*

California already has the most variable precipitation in the continental United States, requiring the state to be prepared to weather both multi-year droughts and severe episodic floods. A suite of studies conducted by the PPIC Water Policy Center on lessons from the 2012–16 drought—the hottest and most severe in modern times—provides insights into drought vulnerabilities and priorities across four sectors—cities, farms, rural communities, and freshwater ecosystems. We found that cities were best prepared, that agriculture was able to adapt through judicious demand management and significant extra groundwater pumping, and that both rural community drinking water systems and freshwater ecosystems were least prepared, and most vulnerable, to extreme drought. As the climate continues to change, several factors will require the state's water policies and management strategies to further adapt to protect the economy and vulnerable communities and ecosystems. Models project continued warming of air and water temperatures, reduced mountain snowpack (now an important source of seasonal water storage), shorter wet seasons, and more intense precipitation variability—with wetter wet years and drier dry years (Ullrich et al. in review, Swain et al. 2018). These changes will put additional pressures on the water system—increasing the water demands for farmland and landscape irrigation, and making it more challenging to balance the competing uses of aboveground reservoirs for urban and farm water supply, cold water for fish, and flood protection. This presentation will describe recommendations on four overarching actions needed to build resilience and reduce vulnerability to droughts of the future: developing more robust drought plans, upgrading the water grid—including above and below-ground storage and to accommodate changing patterns of runoff, updating water allocation rules to facilitate flexible responses to scarcity, and finding the money to address funding gaps in vulnerable sectors. Some of these reforms can be accomplished by building on adaptation work already underway, and others will require a change in course. Successful implementation of the state's Sustainable Groundwater Management Act, adopted in 2014 at the height of the last drought, will be central to success.

**162 Evaluating consequences of POTW effluent in US streams during low flow events**

*J. Rice, University of North Carolina at Charlotte*

Each day over 14,000 publicly owned treatment plants release 87 million $m^3$ of treated wastewater into the nation's freshwater supply, making publicly owned treatment works (POTW) discharges one of the highest inputs of anthropogenic loadings to natural waters. Treated wastewater plays many roles in the U.S.'s aquatic environment and surface water portfolio. Persistent wastewater pollutants potentially pose environmental and human health risks, however treated wastewater can also serve as a primary source of streamflow which in turn can be critical in maintaining stream ecosystems. In this study, a recently-developed national-scale model (DRINCS) was applied to estimate POTW contribution to the loading of contaminants of emerging concern within U.S. surface waters during low streamflow events. Our results indicate streams are vulnerable to endocrine disrupting compounds (EDCs) during periods of low streamflow. And the dependence of contaminant concentrations on streamflow conditions highlights the role that streamflow conditions can play when analyzing surface water samples for EDCs and other wastewater contaminants. This work demonstrates how modeling efforts can be a good accompaniment to environmental sampling, by identifying stream locations where contaminants are expected to be high, signaling where further chemical analysis is warranted.

**163 Assessment of the use of dry wells to infiltrate urban runoff to recharge the aquifer: The Elk Grove Dry Well Study**

*B.S. Washburn, OEHHA / Cal EPA / Ecotoxicology Program; E.C. Edwards, Lawrence Livermore National Laboratory; C. Bowles, cbec eco-engineering; G. Fogg, T. Harter, UC Davis / Land, Air, and Water Resources; B. Lock, OEHHA / Cal EPA / Air, Community, and Environmental Research Branch; C. Nelson, Unico Engineering*

Water reuse is a strategy for optimizing water resources in the face of the changing hydrology associated with climate change in California and elsewhere. One approach involves using dry wells to infiltrate urban stormwater (SW) to recharge the aquifer. However, concerns exist regarding the protection of groundwater quality and drinking water. The Elk Grove Dry Well Study in vestigated this risk by constructing two pilot dry well with pretreatment and a network of monitoring wells, one in a residential neighborhood and a second in a large parking lot for the bus fleet in Elk Grove. Stormwater runoff was collected during 5 rain events and groundwater collected shortly thereafter. Over 200 contaminants were analyzed. Vadose zone modeling was performed to estimate the fate and transport of contaminants found in SW as well as emerging pollutants over a 1000 year timeframe. Data was analyzed using non-parametric statistics, comparing SW before and after pretreatment, in the vadose zone, and in upgradient and downgradient water table wells. Contaminants such as aluminum and bifenthrin that were detected at elevated concentrations in SW were present in low concentrations or not found in groundwater. In many cases, the concentrations of pollutants were no different in upgradient and downgradient wells, suggesting that dry wells were not the source of the contaminants. In contrast, pollutants present at elevated concentration in groundwater, such as chromium or arsenic, were very low or not detected SW. Results of vadose zone modeling suggested that most pollutants would not be quantifiable within the modeling timeframe. Exceptions were two water soluble pesticides, imidacloprid and fipronil. These pesticides are weakly sequestered in the vadose zone and unlikely to be effectively contained by pretreatment. However, their concentrations at the water table after 500 years were 2-fold (fipronil) and 400-fold (imidacloprid) below the health protective concentrations, therefore would pose little risk to human health. Lastly, an extensive review of the literature found little evidence to suggest that the use of dry wells led to a systematic contamination of groundwater. We concluded that dry wells can be safely used to recharge the aquifer if pretreatment and other reasonable safety measures, related to siting and management, are employed.

### 164 Climate change impacts on agriculture in California

*T. Pathak, UC Merced*

California produces more than 400 types of commodities and is a global leader in production of various fruit and nut crops as well as vegetables. With 77,500 farms and 3.8 million ha of irrigated cropland, California generates an overall agricultural production value of $50.5 billion. Despite being the world leader in agricultural production, California agriculture is vulnerable to current and future impacts of climate change. We carried out a detailed review of trends in temperature, precipitations, snowpack, and extreme events including drought and heat waves and consequent impacts on California agriculture. The range of studies on trends and impacts of climate change on California agriculture justifies the importance of enhancing the adaptive capacity of agriculture to climate change impacts, increased research effort to agricultural adaptation to water shortages, and effective stakeholder engagements.

### 165 Nitrate: A Global Water Challenge & Critical Element in Food, Water, Energy Systems

*P. Westerhoff, Arizona State University / School of Sustainable Engineering and The Built Environment*

Nitrogen fertilizer is essential to high productivity agriculture (the Green Revolution); for at least 1/3 of the world's population, N-based fertilizer is the difference between malnutrition and an adequate diet. Industrial production of ammonia ($NH_3$) from di-nitrogen ($N_2$) in the atmosphere via the H-B process consumes 1-2% of the world's annual energy. Loss of N from the food system is a serious environmental problem that leads to atmospheric pollution, dead zones, and eutrophication of freshwater and marine ecosystems. Overall, human use of N-based fertilizers in food production has altered the natural N cascade (i.e., sequential transfer of N between ecosystems). One consequence has been the accumulation of nitrate in aquifers beneath recent and current agricultural areas, which is now needed as a potable water supply. This presentation will describe emerging technologies suitable for removing, rather than just separating, nitrate from drinking water. The focus will be on non-biological treatment processes, because these offer tremendous flexibility and ease of operation.

### 166 A Tiered Framework to Evaluating Risks of Emerging Contaminants from Water Reuse in Agroecosystems

*J. Gan, University of California, Riverside / Environmental Science; Q. Fu, Eawag, Swiss Federal Institute of Aquatic Science and Technology / Department of Environmental Chemistry; C. Sun, UC Riverside / Environmental Science; S. Dudley, UC Riverside / Environmental Toxicology*

Irrigation with treated wastewater and application of biosolids or animal waste introduce numerous contaminants of emerging concern (CECs) into agroecosystems. Accumulation of CECs in food produce constitutes a potential food safety and human health risk. However, a comprehensive risk assessment and management of CECs in food-production systems, i.e., agroecosystems, is constrained by multiple factors, the utmost of which is the enormous number and diverse characteristics of CECs. Here we summarize the current knowledge on accumulation of CECs in plants including vegetables from irrigation of treated wastewater under hydroponic and field conditions. We then delineate processes contributing to the ultimate accumulation of CECs in edible tissues in the soil-plant continuum. We further outline a tiered framework as the path forward to prioritize CECs that may have a high risk for accumulation in food produce. This tiered approach is valuable as it helps us focus our research efforts only on the priority CECs and thus maximizes the use of our limited research resources to tackle this urgent issue.

### 167 Exploring the Interdependencies Between Water, Energy and Climate

*V. Tidwell, Sandia National Laboratories; N. Voisin, Battelle Pacific Northwest Laboratories; J. Macknick, National Renewable Energy Laboratory; E. Yang, Lehigh University*

Energy production withdraws more water than any other sector in the U.S. Water is used to cool thermoelectric power plants, produce hydropower, process fuels and extract energy minerals. Water is also highly dependent on energy for pumping, treatment and distribution. Climate challenges these interdependencies by changing the timing and intensities of stream flows, increasing water and energy demands, changing water quality and encouraging use of non-potable waters requiring increased treatment and transport. Planning and management of this complex interplay is further complicated by uncertainties in future climate, resource demand, technology and policy. In efforts to explore the sustainability and resilience of these interdependent systems an integrated mulit-sector, multi-scale modeling effort is being pursued. Multi-sectoral modeling involves the linking of the physical system (Earth System Models and surface hydrology) with the engineered (reservoir routing and electric dispatch) and human systems (Agent Based Modeling). These coupled systems of models are reproduced at the local, water basin and regional scales. Specifically, comparative analyses link case studies of the San Juan River (northwestern New Mexico), Colorado River Basin and the Western U.S. Numerical experiments explore how system vulnerabilities evolve and how their unique pathways differ not only by the impact metric considered but also according to the region and scale of analysis. Recently, studies have been initiated to consider the efficacy of competing response actions in terms of alternative water supplies, advanced technology, policy and human behavior. *This research was supported by the U.S. Department of Energy, Office of Science, as part of research in Multi-Sector Dynamics, Earth and Environmental System Modeling Program. Sandia National Laboratories is a multimission laboratory managed and operated by National Technology and Engineering Solutions of Sandia, LLC, a wholly owned subsidiary of Honeywell International, Inc., for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-NA-0003525.*

**168 Perspectives from California Water Resources Control Board Member Tam Doduc**

*T. Doduc, California Water Resources Control Board / Board Member*

The California Water Resources Control Board and the Regional Water Quality Control Boards are key parts of the California water system. I will discuss some of our current efforts and thoughts. Integrated efforts on water supplies and management. The Boards support strategies to increase resilience, such as water conservation and efficiency, investments in local water supply development, water recycling, storm water capture and use, increased groundwater recharge, surface storage, and conjunctive use water management, watershed and meadow restoration to enhance natural "above the dam" storage, and groundwater replenishment and sustainable management. The human right to water. For millions of Californians, drinking water costs are high. Also, many residents do not have access to clean and reliable drinking water. Climate change could cause additional challenges to water quality, water supplies, and drinking water and wastewater infrastructure. Disadvantaged communities that already have problems in securing safe water and sanitation will likely have difficulty adapting. The Boards' efforts include the Low-Income Rate Assistance Program, programs of the Office of Sustainable Water Solutions, support for additional consolidation of small drinking water systems that serve disadvantaged communities, and funding efforts. Sustainable groundwater. The Sustainable Groundwater Management Act (2014) will be an important framework for water-related climate adaptation. Moving to sustainability is a long-term effort: groundwater sustainability agencies are required to consider a 50-year "planning and implementation horizon." The Boards plan to facilitate good governance and good outcomes in a range of ways. Water rights. California's approach to water rights is unique relative to other western states, in that the state combines a traditional riparian system (proximity to a water course) with an appropriative (first-in-time, first-in-right) system. This dual system has led to challenges in water rights administration and requires early planning and scenario analysis for water users to obtain new water right appropriations. At present, new appropriations do not consider future climate change scenarios or what climate change will mean for future water availability. Wrestling with the question of climate change and future water availability will require concerted and long-term engagement with the public and the Legislature.

## One Health in Action – Tangible Applications for Problem Solving and Coalition Building

**169 Application of multidisciplinary sciences within USGS toward environmental health goals**

*P. Bright, US Geological Survey / Environmental Health; D.E. Tillitt, US Geological Survey / Columbia Environmental Research Center*

One Health recognizes that there are direct linkages between the environment and the health of humans and other organisms. A key aspect of One Health is understanding how earth processes, environmental factors and human activities interact to influence exposures to microbial, inorganic, and organic contaminants from geologic, anthropogenic, and disaster sources. Moreover, it is critical to understand the health impacts resulting from these exposures. There are many well-known examples of how exposures to contaminants can lead to disease. Many vector-borne and zoonotic diseases are also known to be influenced by environmental conditions, and measures to mitigate disease exposures (such as pesticide usage) can, in turn, have potential health implications. Yet there are still significant gaps in our scientific knowledge; filling these gaps requires truly transdisciplinary collaborations involving a broad spectrum of disciplines (including but not limited to earth, environmental, biological, ecological, social, engineering, and health sciences). This presentation will highlight current US Geological Survey science activities focused on fish and wildlife toxicology research with potential human health implications, collaboration between human health and wildlife researchers, the impact of ecosystem services on health, and the health impacts of environmental disasters.

**170 Toxicological effects of anabaenopeptins and cyanopeptolins to a model organism the nematode C. elegans**

*K.A. Lenz, UWM-ZSPH / Environmental Health Sciences; T.R. Miller, H. Ma, University of Wisconsin Milwaukee*

Cyanobacterial blooms represent a significant risk to environmental, ecological, and human health due to their production of toxic and/or bioactive secondary metabolites, or cyanopeptides. Anabaenopeptins and cyanopeptolins are cyanopeptides that have been increasingly detected in drinking water, food sources, and medicinal agents and supplements at concentrations exceeding toxicity levels for other cyanopeptides (such as microcystins). Yet, their potential environmental and human health implications are not well understood. Here we assessed the toxicological effects of three anabaenopeptins (AP-A, AP-B, and AP-F) and three cyanopeptolins (CYP-1007, CYP-1020, and CYP-1041) to a model organism the nematode *Caenorhabditis elegans* (*C. elegans*). Examined toxicity endpoints included reproduction, hatching time, growth rate, lifespan, and age-related vulval integrity. Microcystin RR (MC-RR) and microginin 690 were also included in the study for comparisons. Eggs or young adult worms were exposed to an environmentally relevant concentration of these cyanopeptides (10 µg/L) and observed for potential toxicological effects through the life course of the worms. APs showed the greatest toxicity among all cyanopeptides tested, indicated by reduced reproduction, delayed hatching, reduced growth rate, shortened lifespan, and severe vulval integrity defects. The three APs (10 µg/L) decreased worm reproduction, indicated by the number of progeny per worm, by 23%-34% compared to the control. APs also induced an average of 1.6 hr delay in hatching, reduced the average growth rate by 12.3%-15.7% after 24hrs, and shortened the lifespan by 5 days (corresponding to a 30% reduction in lifespan). The most remarkable impact of these APs was the Avid (age-related vulval integrity defect) phenotype, where more than 95% of exposed worms developed an Avid phenotype, compared to a less than 10% incidence in control worms. Cyanopeptolins (CYPs) displayed similar toxicity as MC-RR, and Microginin 690 was the least toxic. These findings suggest that APs and CYPs may pose significant health risks to aquatic organisms, and potentially human health. Overall this study suggests more toxicological studies of these cyanopeptides using different species across different trophic levels are needed to gain a thorough understanding of their potential impact on ecological systems and human health.

**171 Concentrations and risks of metal contamination from lead shot in a Hawaiian green sea turtle (*Chelonia mydas*) foraging aggregation and their habitat**

*K. Shaw, Texas Tech University / Environmental Toxicology; G. Balazs, T. Jones, National Oceanic and Atmospheric Administration / Pacific Islands Fisheries Science Center; H.W. Lynch, The Nature Conservancy of Hawaii; D. Klein, Texas Tech University / Department of Environmental Toxicology; J.M. Lynch, National Institute of Standards and Technology / Chemical Sciences Division*

The Kaimalino neighborhood in Kailua Bay, Hawaii, located on windward Oahu, was once the home of the Honolulu Skeet Club. During the 40 years the skeet club was active, lead (Pb) shot was used resulting in an accumulation of these pellets on and near the coastline. The pellets slowly degrade and release Pb, arsenic (As) antimony (Sb) and tin (Sn) and put organisms living, breeding, and foraging in this area at risk of heavy metal accumulation and toxicity. Signs at the public access point currently warn that Pb and As found in pellets along the shoreline may be harmful to children if swallowed. The Honolulu Skeet Club is listed as an active superfund site by the Environmental Protection Agency, but is not on the national priorities list. One species found in Kailua Bay is the threatened green sea turtle (*Chelonia mydas*). Green sea turtles studied since 2000 along the shoreline of Kaimalino neighborhood at the mouth of the Kawainui Marsh are highly resident, with 75% of turtles re-captured during sampling events spanning three years, 2011-2013. This high site fidelity allows sea turtles to serve as bioindicators of contaminants in Kailua Bay. In a preliminary study, the maximum blood lead concentration in Kailua Bay turtles was 140 ng/g. This exceeds the Center for Disease Control children blood Pb reference level of concern of 40 ng/g by more than three times. An additional 33 turtles have been sampled for this study. Ten of these turtles were sampled twice, providing a temporal history of blood and scute Pb, As, Sb, and Sn concentrations, while the remaining 23 turtles were sampled once. Some of the turtles sampled are new recruits from the pelagic habitat to the bay with a straight carapace length (SCL) of approximately 40 to 45 cm. Larger turtles that have been caught over multiple years are likely residents of the bay, and potentially exposed to the Pb shot for multiple years. Sediment, algae, water, and lead shot samples taken from Kailua Bay will allow us to trace the metals through the ecosystem and provide additional data for possible human exposure.

### 172 Reciprocating Relationships to Wellness

*T. Godfery, Avicennia Education / School of Undergraduate Studies; H. Hireme, Te Whare Wananga O Awanuiarangi / School of Undergraduate Studies*

In his essay titled The Land Ethic, Aldo Leopold (1949) states "*There is as yet no ethic dealing with man's relation to land and to the animals and plants which grow upon it. Land, like Odysseus' slave-girls, is still property. The land-relation is still strictly economic, entailing privileges but not obligations.*" Whilst this statement remains true throughout much of the world, conversely, the concept of land as property is disturbing to many indigenous communities. An advanced ethic of environmental care has developed over time, with indigenous peoples amongst continuing practitioners of this ethic. An ethic premised upon interconnectedness and interdependence, which includes relationships of reciprocity and respect. Notwithstanding the integrative nature of the One Health model, caution is however necessary when including elements based upon indigenous relationships. Practitioners must ensure these elements maintain integrity, and are not subsumed by scientific or technical components. This paper discusses the value of reciprocal and respectful relationships with environment, proposing such relationships are vital to achieving wellness. Discussion also highlights potential contributions to the One Health Model.

### 173 Examining the relationship between chemical/non-chemical stressors and inherent characteristics to explain disparities in children's mental health

*F. Nilsen, USEPA / National Exposure Research Lab; N.S. Tulve, US Environmental Protection Agency / Office of Research and Development / National Exposure Research Laboratory*

Children may be more vulnerable to the combined interactions of chemical and non-chemical stressors from their built, natural, and social environments when compared to adults. Up to 20% of children are diagnosed with a mental illness annually in the United States with a large number not receiving adequate treatment. This project aims to elucidate the links between chemical and non-chemical stressors and inherent characteristics on children's mental health outcomes using our multifactorial conceptual framework. Serotonin is one of the most important neurotransmitters related to mental health. Monoamine oxidase A (MAOA) aids in serotonin uptake and has two genotypes that affect its enzymatic activity (low; L, and high; H). MAOA-L carriers are more frequently diagnosed with behavior and emotional disorders. When children are exposed to cigarette smoke, bisphenol A (BPA), or organophosphate pesticides, MAOA activity is inhibited. Non-chemical stressors, such as traumatic childhood experiences, ethnicity, and lifestyle factors, complicate the relationship between genotype and chemical exposure. But, the co-occurrence among outcomes between chemical exposures, genotype, and non-chemical stressors with MAOA-L suggest that mental illness in children may be caused by multiple interacting factors. The interacting factors described here are being assessed by a state-of-the-science review to clarify the relationship and discrepancies observed. Preliminary results show that exposed animals acquire behaviors characterized by MAOA-L. In humans, adult non-chemical stressor exposure is correlated to many MAOA-L associated mental health outcomes, and MAOA-L children experiencing trauma typically develop at least one mental disorder. Chemical exposure is not considered in most epidemiological analyses, but may be a contributing factor. Population genetics also play a role in behavioral outcomes, but results often contradict what is established for MAOA-L, with epigenetic changes adding another layer of complexity. If a relationship is observed across all factors, a meta-analysis using mental health, traumatic experience, and genetic data will be conducted to better understand the causes of mental illness in children. An improved understanding of inherent vulnerability and the onset of mental illness may provide better intervention and treatment options to children in need.

### 174 A Health and Ecological Systems Framework for Vector-Borne Diseases in Puerto Rico

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Environmental, social and economic factors can influence public health, but the interactions can be difficult to understand and anticipate. A systems context, that is, a conceptual approach that simultaneously considers environmental, social and economic factors, is essential. Previously in the One Health session, we described a systems framework originating from an environmental context (DPSIR—Driving force, Pressure, State, Impact and Response) that was adapted to include social and economic aspects of human activity and behavior. The resulting EcoHealth DPSIR was illustrated using vulnerability and effects of asthma. The concept is further illustrated by examination of recent research in the San Juan metropolitan area of Puerto Rico in relation to vector-borne (mosquito) Zika and dengue diseases. Two studies demonstrated correlation of environmental factors, such as wetland area, nutrient enrichment, rainfall and flooding with the capacity of mosquito populations to carry virus and subsequent disease incidence in humans. Socio-economic factors, such as income level, moderated the influence of environmental factors on disease incidence in humans. The resulting relationships emphasize the importance of factors like equity, governance and personal choices in public health; and the consequent impacts of disease on human well-being and economic production.

### 175 One Health approach to addressing a mysterious kidney disease

*R. Babich, University of Maine; A. Massarsky, Duke University / Nicholas School of the Environment; Y. Sugano, Harvard University; K. Wanigasuriya, P. Manage, University of Sri Jayewardnepura; R.T. Di Giulio, Duke University / Nicholas School of the Environment; I. Drummond, Harvard University; N. Jayasundara, Universiy of Maine / School of Marine Sciences*

A mysterious chronic kidney disease (termed as chronic kidney disease of unknown etiology – CKDu) has emerged across certain farming communities across the world, including in South Asia. Notably, this disease is a major epidemic in rural agricultural communities in Andhra Pradesh, India and in several parts of Sri Lanka. In affected communities in Sri Lanka, 20% of the adult population is diagnosed with the disease. The primary obstacle to mitigating CKDu is the unknown etiology of this condition. Exposure to environmental contaminants (e.g., heavy metals and pesticides) through ingestion is thought to contribute to CKDu. However, a specific role for individual chemicals has been repeatedly tested without compelling positive results. Alternatively, synergistic and interactive toxic effects of chemical mixtures are hypothesized to cause CKDu. Nonetheless, despite being much more environmentally realistic, evaluating the role of chemical mixtures in CKDu as well as in other chronic diseases remains a major challenge. Here, to gain insights into adverse health effects of exposure to chemical mixtures in regions affected by CKDu, we integrated laboratory exposure studies and sentinel species analyses. For laboratory studies, we used zebrafish *Danio rerio* embryos to evaluate toxicity of chemical mixtures derived from lakes and drinking-water wells from CKDu regions. We examined exposure effects on survival rates, teratogenicity, kidney specific oxidative stress, kidney development, mitochondrial function, and kidney specific gene expression changes. To complement our laboratory studies, we are taking a One Health approach to examine teleost kidney health in fish inhabiting lakes from affected regions. Chemical analyses of mixtures derived from these regions showed that cadmium, arsenic, and lead are present in these mixtures, but at levels well below current safety thresholds. Despite low levels of these chemicals, toxicity studies indicate that chemical mixtures derived from lakes and wells from CKDu affected regions are toxic to mitochondria and induce kidney specific oxidative stress as well as gene expression changes. Importantly, including sentinel species into our studies is providing a framework for integrating a One Health approach in evaluating and predicting adverse health effects of long-term exposure to chemical mixtures.

**176 Translational Science Research and One Health**

*C. Baghdikian, US Environmental Protection Agency / Office of Research and Development / National Health and Environmental Effects Research Laboratory*

There are several complex or “wicked” problems related to air, water, and soil that impact ecosystems and communities both domestically and abroad. Many of these problems are aligned with USEPA’s mission to protect human health and the environment. Addressing these wicked environmental problems requires interdisciplinary approaches that engage multiple stakeholders to fully characterize the nature of the problems and their potential solutions. To improve research planning and direct applicability of research to stakeholder needs, ORD recently launched a Translational Science initiative. Translational science is the study of translational research – how to produce and deliver science that directly informs decisions or actions, e.g. how to move from science to solutions. Translational research approaches emphasize stakeholder engagement throughout the entire research process, starting with problem formulation and informing all elements of research planning, implementation, dissemination, and evaluation. This initiative consists of a three-pronged approach: 1) apply principles of translational research broadly across ORD’s six research programs; 2) conduct two translational science pilots to advance the science of how we do translational research; and, 3) prepare case studies of translational research approaches in ORD to begin to extract successful methods. This session will describe some of the processes and findings of each of these activities as they relate to the interdisciplinary, multi-stakeholder nature of the problems that face the One Health community at SETAC.

# Cradle to Grave Impacts of Nanotechnology

### 177 Comparative Life-cycle Analysis of Nitrate and Perchlorate Technologies for Microbial Sulfate Reduction Inhibition in Offshore Oil Fields

*B. Neupane, Energy Biosciences Institute / University of California, Berkeley; C.D. Scown, Lawrence Berkeley National Laboratory / Energy Analysis & Environmental Impacts*

Hydrogen sulfide ($H_2S$) generation by sulfate-reducing microorganisms (SRM) presents human health, environmental, and economic risks in the offshore oil production industry. Corrosion due to hydrogen sulfide ($H_2S$) generation is the leading cause of oil industry infrastructure failure with high economic consequences, estimated to range billions of dollars per year. This study compares the life cycle environmental performance of existing nitrate and proposed perchlorate-based technologies to prevent $H_2S$ formation and evaluates environmental impacts of oil leaks and spills due to corrosion damages at offshore oil wells. We developed a detailed comparative life-cycle inventory to examine the environmental impacts, including GHG emissions and ecotoxicity impacts of sodium perchlorate and calcium nitrate production, and of their subsequent use in an offshore oil field to prevent hydrogen sulfide ($H_2S$) generation. We also report the environmental damages in offshore oilfield due to $H_2S$ generation and potential benefits of sodium perchlorate treatment in preventing such impacts. We used the real field data (i.e., oil production rate, $H_2S$ generation, nitrate injection rate, etc.) based on an offshore oil field in Europe. Using the best available data, we found that a sodium perchlorate treatment strategy can offer substantial environmental and economic savings relative to calcium nitrate at eliminating $H_2S$ corrosion-related oil well shutdowns. Our results suggest that, although both the technologies have comparable GHG emissions across the supply chain, other impact categories such as acidification and toxicity impacts are improved with sodium perchlorate treatment. Given the uncertainty with the several process parameters, detailed sensitivity analysis is conducted to better understand the potential impact on the environment across the supply chain.

### 178 Modeling Spatial Dimensions of Life Cycle Nanomaterial Emissions

*E. Moore, C.W. Babbitt, Rochester Institute of Technology / Golisano Institute for Sustainability; B. Tomaszewski, Rochester Institute of Technology / Computing and Information Sciences; A.C. Tyler, Rochester Institute of Technology / Environmental Science; G. Gaustad, Rochester Institute of Technology / Golisano Institute for Sustainability*

As the demand for higher performance products rises, novel materials like carbonaceous nanomaterials (CNMs) are increasingly integrated into different consumer and industrial products, which affects what part of the life cycle CNMs could be released. It is important to identify the geographic location of production and potential releases to inform knowledge gaps for life cycle impact analysis of emerging materials. Past literature has tried to estimate the locations and concentrations of potential nanomaterial releases throughout a product's life cycle. However, national and global average approaches may underestimate accumulation of releases if they concentrate in a certain area. Since there are varying vulnerabilities depending on the release site (i.e. interaction with threatened habitats, nutrient cycling changes, and trophic level impacts), the location matters and thus informs the magnitude of risk. Because predictive modeling is necessary to estimate likely CNM release patterns and material flows (manufacturing siting, regional adoption of CNM-enabled products, and ecosystem geography), ArcGIS modeling methods were adapted to analyze potential releases from two case study CNM-enabled products. An integrated modeling tool that calculates realistic spatial concentrations of CNMs released from a commercial product (electric vehicle lithium-ion batteries) and an emerging product (organic photovoltaic cells) was developed and compared to empirical ecotoxicity data to determine if there is a chance of emitting high enough concentrations of CNMS that would cause environmental impacts in a targeted siting scenario. Preliminary results show potential CNM exposure from production facilities and disposal sites that are within buffer zones of the Great Lakes watershed and wetland habitats. This integrated modeling and empirical approach is novel to the nanomaterial literature and can help minimize risks to vulnerable ecosystems.

### 179 Estimated release of titanium dioxide from personal care products entering wastewater treatment plants

*F. Wu, University of Wisconsin-Madison / Chemical, Biological and Environmental Engineering; A. Hicks, University of Wisconsin – Madison / Civil and Environmental Engineering; M. Seib, Madison Metropolitan Sewerage District*

Although current regulation indicates titanium dioxide ($TiO_2$) is non-toxic compound, $TiO_2$ nanoparticles (NPs) are considered as an emerging environmental contaminant. Research indicates up to 30% of $TiO_2$ found in personal consumer products (PCPs) are present at nano-scales (one or more dimensions is equal to or less than 100 nm) to achieve applications such as UV protection, coloring, catalysis, and greater sizes serve as a texture modifier in food and PCPs. With the high quantity and wide applications in PCPs, $TiO_2$ is dispersed during use, then entering the waste water treatment plant (WWTP) and ultimately the environment. Traditional studies by directly measuring the $TiO_2$ concentrations in WWTP is localized and difficult to represent $TiO_2$ concentration at large scale. This work utilizes a citizen science approach to generate information as to the quantity of $TiO_2$ entering the WWTP to enhance the knowledge on the life cycle of $TiO_2$ at usage stage, refine the estimates of $TiO_2$ entering the WWTP as a function of the use of PCPs to determine future potential adaptions. In addition, the current study aim is to develop a PCP inventory to list products contain $TiO_2$ (potentially nano-$TiO_2$) for future research and benefit regulators and industry. The objectives are accomplished by surveying PCPs in households from the sewerage district and estimating $TiO_2$ emission at individual household level from use of PCPs. In the survey, information is collected on the brand, number of products, and if contain $TiO_2$, from most commonly used PCPs in the household (such as toothpaste, shampoo, skin care, sunscreen, etc.) along with the demographic information. Survey results are combined with the daily usage and amount of $TiO_2$ in each product to estimate the human exposure and the contribution of $TiO_2$ release to the WWTP from the use of PCPs. $TiO_2$ concentrations in wastewater samples are measured by ICP-MS. Results identify sunscreen to be the product contains the most $TiO_2$. A citizen scientist roundtable meeting will be held to present the results and gain insight from the citizens to future solutions as the next phase. The information generated throughout this study is useful to prioritize future research, such as the life cycle of $TiO_2$ containing consumer products, predict the fate and transport of $TiO_2$ released from PCPs, and possibly refine future regulations based on the applications and pathways that are most likely to impact human health and the environment.

### 180 Insights from life cycle assessment of nano-silver enabled consumer products

*A. Hicks, University of Wisconsin – Madison / Civil and Environmental Engineering*

Silver has been utilized for its antimicrobial properties since antiquity. Nano-scale silver (nAg) with dimensions of less than 100 nanometers (nm) is one of the most commonly used nanoparticles in consumer products due to its antimicrobial efficacy which has been found to be enhanced compared to bulk silver. These products include textiles, food storage containers, sprays, toothpaste, among others, with the goal of providing an antimicrobial characteristic to these products. A major question has been whether the benefits bestowed by the addition of nAg to consumer products outweighs the environmental costs. Life cycle assessment (LCA), a systematic tool for determining the environmental impacts of products and processes throughout their lifetime, has been applied to evaluate the environmental impacts of nAg enabled products. In most instances the additional environmental burden due to the incorporation of nAg is relatively small, however, that is not universally true across all products and environmental impact categories. This work utilizes life cycle assessment to frame the discussion as to the relative environmental costs (or impacts) and benefits (such as antimicrobial efficacy) enabled through the addition of nAg to consumer products, highlighting multiple consumer products. The findings suggest that although the additional environmental impact is often small, in some instances, due to the antimicrobial ineffectiveness of the nAg, that the nanoenabling is not worthwhile. This may serve as the starting point for a framework to determine when the benefits of nano-enabling consumer products with nAg outweigh the costs.

**181 Nanoparticle Detection in Environmentally Relevant Matrices and Biota Using Single Particle ICP-MS**

*M.E. Ellefson, 3M Company / 3M Environmental Laboratory; C.Q. Chan, 3M Company / Corporate Reseach Analytical Lab; B.T. Mader, 3M Company / Environmental Laboratory*

Engineered nanoparticles frequently are used as additives to consumer products to enhance product properties. Understanding the environmental fate and impact of engineered nanoparticles relies on the ability to characterize them at relatively low levels in complex media and biota, which often contains additional naturally occurring nanoparticles. We present the application of a recently developed analytical technique which utilizes elemental analysis of individual nanoparticles using inductively coupled plasma mass spectrometry (spICP-MS) to characterize gold and silver nanoparticles in environmentally relevant water samples including ground water, industrial wastewater, and ecotoxicology test media. The method is capable of detecting nanoparticles of 10-100 nm diameter at part-per-trillion (ppt) levels in environmentally relevant aqueous solutions. Following instrument characterization, the spICP-MS was used to assess the fate of gold nanoparticles in a solution containing daphnia magna. Insights into dose verifications obtained by spICP-MS will be discussed as well as the method's performance and the advantages of using spICP-MS for dose verification and determining environmental fate of gold and silver nanoparticles in complex environmentally-relevant sample matrices.

**182 Altering silver nanowire diameter for intelligent re-design and recycling to improve the safety and sustainability of touchscreen technology**

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Silver nanowires are replacing indium tin oxide in touchscreen applications, but it is not clear if silver nanowires will exhibit behaviors that are different from their spherical counterparts in biological systems. This project focuses on human and environmental impacts of silver nanowires (AgNWs) as a consequence of their synthesis and application in touchscreen technologies. The goals of this project are to determine if the environmental fate and impacts of AgNWs differ from spherical silver nanoparticles, if AgNWs are released from touchscreens during aging or environmental exposure, and if silver can be reclaimed from touchscreens after disposal. This project is part of a transnational collaboration that highlights multi-disciplinary perspectives to inform risk management strategies in the U. S. and Europe. AgNW synthesis and characterization are centralized at the University of the Grenoble Alps, France to decrease AgNW variability among participating laboratories. Results indicate that AgNW diameter is a key factor that influences AgNW toxicity to humans and aquatic organisms. Thick AgNWs (90 nm diameter; 10 µm length) induced significantly more cytotoxicity compared to thin AgNWs (30 nm diameter; 10 µm length), and this was determined in multiple cell lines (mouse fibroblasts, rainbow trout gill and gut cells) looking at a number of endpoints (ATP production, membrane integrity, ROS production, mitochondrial membrane potential, etc.). X-ray microscopy images reveal mechanical crumpling of thin AgNWs after cellular uptake, while thick AgNWs maintain their rigid structures. These different mechanical behaviors after cellular uptake can explain their different toxicity in cells, as crumpled AgNWs are less likely to damage intracellular structures. Whole organism assays on rainbow trout embryos and fry indicate some toxicity of AgNWs, but no significant differences between thick and thin AgNWs were observed. Weathering experiments indicate the corrosion of AgNW thin films is possible under certain environmental conditions despite the coating of the AgNW film by a protective resin, and an electrochemical technique for reclaiming Ag from AgNW-enabled technology has shown promising results. Future work will focus on the bioaccumulation and trophic transfer of AgNWs in an aquatic system, and RNAseq to further evaluate the mechanisms of differential AgNW-diameter related toxicity.

**184 Trophic transfer of differentially charged CeO2 nanoparticles from tomato plants to hornworms**

*J. Li, University of Kentucky / Plant and Soil Science; J. Unrine, University of Kentucky*

The entry of manufactured nanomaterials (MNM) into the food webs has the potential for far-reaching impacts on ecosystems. Our previous studies and those of others have demonstrated trophic transfer of MNMs. However, our knowledge is still incomplete regarding the dietary transfer of MNM along the food chain and the specific role of particle surface chemistry. This study investigated the dietary uptake and elimination of polymer-coated $CeO_2$ nanoparticles (NPs) carrying a net positive, neutral or negative charge in a simulated food chain consisting of the tomato plant (*Solanum lycopersicum* cv Micro-Tom) and the tobacco hornworm (*Manduca sexta).* Hydroponically cultivated tomato plants were exposed to the three different types of $CeO_2$ NPs at non-toxic concentrations for 14 days. Freshly cut leaves were used to feed the hornworms. After 7 days of feeding allowing the uptake of $CeO_2$ NPs from the food, elimination of $CeO_2$ NPs occurred by feeding leaves from untreated plants to the hornworms. Randomly selected hornworms were sacrificed and analyzed at various time points throughout the process to obtain a time course of Ce dynamics. Despite no observed overall biomagnification across trophic levels, these differentially charged CeO2 NPs had higher bioaccumulation factors than that of ionic $Ce^{3+}$. The uptake-elimination dynamics were influenced by the surface charge of the NPs. Positively charged NPs had higher bioaccumulation factors and assimilation efficiencies but lower elimination rate than neutral and negatively charged CeO2 NPs. These results provide important evidence that physico-chemcial properties such as surface charge have a great impact on the trophic transfer and uptake-elimination dynamics of MNM within terrestrial food chains.

## Micro-organisms and Biopesticides in Crop Production: Use and Environmental Safety Considerations

**185 Future products of biotechnology and needs for risk analysis science: Findings and recommendations of a National Academies of Science study**

*S.P. Bradbury, Iowa State University / Natural Resource Ecology and Management*

In July 2015, the Office of Science and Technology Policy in the Executive Office of the President initiated an effort to modernize the U.S. government's regulatory system for biotechnology products. As part of this effort, a committee of the National Academies of Sciences, Engineering, and Medicine was tasked to forecast likely future products of biotechnology over the next 5-10 years and the scientific capabilities, tools, and expertise needed by the U.S. regulatory agencies to ensure efficient and sound risk analyses. The committee's information gathering efforts included dialogue with the U.S. Department of Agriculture, Environmental Protection Agency, Food and Drug Administration, and other Federal agencies; public meetings; and review of the literature and recent Academies' reports on gene drives, genetically-engineered crops, and the industrialization of biology. The committee evaluated technological, economic and social drivers associated with products likely to enter the market over the next 5-10 years. The Committee also evaluated whether future biotechnology products could pose different types of risks relative to existing products and organisms. The Committee's report was released in March 2017. The report concluded that the bioeconomy is growing rapidly and the number and complexity of future products, and diversity and number of new product developers, has the potential to overwhelm the U.S. regulatory system. The report provides fourteen specific recommendations for how the U.S. government can improve its capabilities to evaluate future products through advances in regulatory governance and risk analysis techniques. The recommendations fall into three broad themes: a) enhance scientific capabilities and tools in the natural, social and regulatory sciences; b) develop prototype approaches for assessing risks and benefits, with public participation and external peer review, for future unfamiliar and complex products identified through horizon scanning; and c) connect biotechnology research and development pipelines with proactive advances in regulatory science. The full report titled, Future Biotechnology Products and Opportunities to Enhance Capabilities of the Biotechnology Regulatory System, is available at http://nas-sites.org/biotech/

**186 Characterization and manipulation of crop microbiota**

*J. Leveau, University of California – Davis / Department of Plant Pathology*

Plants are colonized by a diverse community of microorganisms known as plant microbiota. The interactions that these microorganisms have with each other and with their plant host determine the health of the plant and in the case of commercially important crops, the yield and quality of the plant product. Using processing tomatoes as an example, this presentation will explore the question of what constitutes a 'healthy' microbiota and what can be done to fix an 'unhealthy' one. More specifically, it will present an in-depth profile of the bacterial and fungal microbial communities in the soils and on the roots of processing tomato plants as it relates to field location and the application of various chemical and biological soil amendments. Also presented will be the results of a study into the utility of bacteria from the genus Collimonas to protect tomato plants from soilborne fungal pathogens.

**187 Global Survey of Microbial Pesticide Regulation and CropLife International 's Microbial Pesticide Safety Framework**

*R. Garnett, CropLife International; M. Herrero, Valent; M. Hey, Monsanto; N. Hubbard, Corteva; R. Kneen, Bayer; M. Liebergesell, FMC; D. Munday, Sumitomo; L.S. Ortego, Bayer CropScience / Environmental Toxicology and Risk Assessment; S. Rutherford, European Crop Protection Association ECPA; J. Vialaneix, BASF; A. Scarr, Syngenta*

Microbial pesticides are a type of product derived from naturally-occurring organisms which are used to prevent, destroy, repel or mitigate a pest. They can be eukaryotic in nature such as protozoa, algae, and fungi or prokaryotic such as Eubacteria and Archaebacteria. They can also be parasitically replicating microscopic elements such as viruses. Microbial pesticides are used throughout the world, but regulation can be quite different country to country. Many countries apply their chemical regulation system to microbial pesticides, but the testing and risk assessment requirements for chemicals are generally inappropriate for the regulation of these products, e.g., the chemical testing guidelines do not include provisions for determining pathogenicity. A few jurisdictions have microbial-specific regulation including US, EU, China, Japan, and Brazil. Other countries may have very little regulation of these products and require little if any environmental safety data or risk assessments. CropLife International developed an environmental safety framework for microbial pesticides in order to aid member companies in evaluating the environmental safety of their products. In this framework, a base set of information is described which can be used to evaluate environmental safety and risk and the need for additional testing. It is recommended that this information may come from reliable and relevant peer-reviewed literature when available *in lieu* of performing additional testing. An appropriate conceptual model also should be used to determine if there is exposure to the compartment of concern; the safety evaluation should focus on organisms that may be exposed. In this presentation, a brief global survey of various environmental evaluation requirements and approaches will be presented. The CropLife International framework will be described focusing on the approach for viable bacterial and fungal microbial pesticide environmental safety evaluation.

**188 Ecological Risk Assessement for Microbial Pesticides in the US**

*S. Borges, USEPA / Biopesticides and Pollution Prevention Division*

The U.S. Environmental Protection Agency (EPA) defines biopesticides as certain types of pesticides that are derived from such natural materials as animals, plants, bacteria, and certain minerals. The EPA recognizes that biopesticides have certain unique properties and benefits, and has developed a specific regulatory approach for these types of pesticides. Microbial pesticides are a category of biopesticides that consist of eukaryotic (e.g., fungi, algae, protozoa) and prokaryotic (e.g., bacteria) microorganisms, as well as autonomous replicating microscopic elements (e.g., viruses). General concepts of ecological risk assessment apply to microbial pesticides, but special considerations must be made in exposure assessment and nontarget testing to account for their potential viability in the environment and pathogenic effects. The EPA has developed specific guidelines for nontarget testing for microbial pesticides, which capture these special considerations and are required according to a tiered scheme. This testing is based on guidelines developed for chemical pesticides, but takes into account the unique nature of microbials in specific methods and/or general approach. While some challenges exist, the EPA's approach has proven to be a viable balance between data needs for risk assessment and regulatory burden.

**189 Considerations for Ecological Testing and Assessment of dsRNA Agricultural Products**

*J. Fischer, Monsanto Company / Global Regulatory Sciences*

The discovery and development of double-stranded RNA (dsRNA) products that confer protection from insect pests through a new mode of action have the potential to provide significant value in agriculture. The sequence specific nature of dsRNA allows these products to target pest species with a high level of specificity, while mitigating risk to non-target organisms. Registration and commercialization of these products requires comprehensive science-based ecological risk assessments to ensure these products are safe for the environment. As is the case with traditional crop protection products, ecological risk from a dsRNA insect control product is a function of potential harm and environmental exposure. Proposed use patterns for a dsRNA product such as expression by a genetically-modified crop or topical application inform the relevant routes and potential duration of exposure. Such considerations in addition to mode of action, activity spectrum and bioinformatics analysis can provide guidance for appropriate non-target organism testing. This presentation will provide examples of how this scientifically relevant information can be used to inform problem formulation and how problem formulation can be used to develop an ecological assessment plan for dsRNA products.

**190 Dissipation of dsRNA in an aqueous system**

*J.R. Coats, Iowa State University / Entomology; C. Wong, V.C. Albright, Iowa State University / Department of Entomology*

The use of double stranded RNA (dsRNA) to induce RNA interference in pests has proven effective, and its potential use to control insect pests is being examined closely. This study examined the persistence of a non-bioactive dsRNA sequence in aquatic systems with water over sediment. Methods: Test systems consisting of purified water and sterilized sediment, sterilized pond water and sterilized sediment, and active pond water over active sediment were all inoculated with the dsRNA applied to the water phase. Samples were extracted via a proteinase K digestion from the sediment and the overlying water separately. Sample systems were allowed to incubate for time periods up to 14 days. Dissipation of dsRNA was determined by QuantiGene® 2.0 Singleplex assay kit (Affymetrix).

The kit was tailored by the manufacturer to match this specific dsRNA sequence. Results/conclusion: For all experimental systems, dsRNA recovery was reduced to background levels by 96 hours post-inoculation. Movement from water into the sediment was minimal, with at most 3% of the initial dsRNA found in the sediment of the system. The half-life of the sequence was similar for all systems, ranging from 56 to 72 hours, which suggests that abiotic factors alone can degrade dsRNA in the environment.

**191 Challenges in Testing Biopesticides in Conventional Laboratory Ecotoxicology Studies**

*H.O. Krueger, EAG Laboratories; D. Brougher, J. Griebel, EAG Laboratories / Aquatic Toxicology*

Standardized testing procedures have long been established for testing aquatic and terrestrial organisms. These tests have been optimized for conventional pesticides where the physical/chemical properties and routes of exposure of the test substances are well understood. The rules for setting test concentrations for conventional tests are well understood, but additional guidance is needed for biopesticides. Quantification and confirmation of the test item is not as straightforward as conventional testing. Microbial materials require plating and results need to be converted from the number of colony forming units to mg product/L. In aquatic testing the limit of solubility is often used to determine the highest test concentration tested. For many biopesticides, solubility does not really apply and the test material may actually grow and increase over the course the test. Another issue is that in aquatic testing solvents may be used to get materials in solution. Such solvents can be highly toxic to biopesticides limiting the use of carriers in test systems. Additionally, after adding a biopesticide to a test system in an aquatic test, either the test item or its carrier often reduces the visibility in test solutions making observations difficult during the test. Dissolved oxygen levels in water can also be of concern and gentle aeration from test initiation is advised. Products that are proteins can denature making it hard to maintain test concentrations. Microbial seed coatings also are challenging. How do you set test concentrations for chronic honey bee tests when the test material will not translocate in plants and end up in nectar or pollen? Another consideration in bee testing is the antimicrobial properties of royal jelly. As was done with conventional materials there is a need to work collaboratively among academia, government, and industry to develop better guidance for testing.

**192 Safety Testing Strategies To Evaluate The Potential Effects Of Biopesticides Used In Crop Protection Products On Aquatic Organisms**

*A.E. Fournier, M.J. Bradley, L. Sayers, R.C. Biever, Smithers Viscient / Department of Ecotoxicology*

Biopesticides as defined by the USEPA are divided into three general categories: biochemicals, which are naturally occurring substances, microorganisms or microbial pesticides, and plant-incorporated protectants (PIPs). The USEPA first issued guidance for testing microbial pest control agents (MPCA) in 1983. While it is recognized that these organisms are unique with specific modes of action, the guidance is vague and has remained relatively unchanged over the last 35 years. Other regions in the world typically look to the US in the testing and risk assessment process for MPCAs and thus better guidance in these regions is not available. The guidance documents are brief and tend to take standard chemical testing principles and procedures, and then applies those strategies to MPCAs. This typically leads to having to make significant modifications to exposure systems used in chemical testing to assure that the MPCAs are dosed properly and stay suspended in the water column, without causing physical effects to the organisms. Additionally, confirmation of this dosing and suspension through chemical specific analysis or microbial evaluation has presented an area for further challenges with little guidance. Another unique challenge in safety testing of MPCAs is that evaluation of pathogenicity is required in addition to general toxicity, thus the exposures need to be longer in duration, with a gross necropsy at the termination of the exposure. We will present an overview of the aquatic testing requirements, identifying the test substance and necessary controls, some of the modifications utilized to manage exposures, challenges in measuring exposure and look at some typical outcomes of aquatic testing with MPCAs. Methodology used in the testing of MPCAs will be compared to methods used in testing biochemicals.

## Birds Under Stress: Impacts of Chemical Exposure and Environmental Changes – Part 1

**193 Spatial differences in mercury exposure in blood and eggs of a declining seabird in Atlantic Canada: Leach's storm-petrel**

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Leach's storm-petrel (*Oceanodroma leucorhoa* = *Hydrobates leucorhous*) is a small seabird species that was listed as globally vulnerable on the IUCN's Red List of Threatened Species in 2016. Breeding populations have declined more than 30% over the last three generations in Atlantic Canada. This migratory seabird species faces multiple stressors in their breeding and wintering habitats. Previous studies had revealed that elevated mercury exposure may be one such stressor. As part of an assessment of the role of methylmercury toxicity in these population declines, we measured mercury concentrations and stable carbon, nitrogen and sulphur isotope ratios in the eggs and blood of adult Leach's storm-petrels at five breeding colonies across Atlantic Canada. Eggs were collected soon after laying in 2016 and 2017. Blood was collected from incubating adults (in different nesting burrows from the egg collections) about a month later. We measured total mercury and stable carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and sulphur ($\delta^{34}S$) isotope ratios in egg contents and whole blood. Geometric mean mercury concentrations in the blood of adult Leach's storm-petrels (n=191) were significantly greater at two colonies around Newfoundland (Baccalieu 1.10 µg/g wet wt. and Gull 0.99 µg/g) than at three colonies in the Bay of Fundy and Scotian Shelf (Country Island 0.83 µg/g wet wt, Bon Portage 0.76 µg/g and Kent 0.41 µg/g). A slightly different spatial pattern emerged from the storm-petrel eggs (n=106), where the geometric mean mercury concentrations were significantly greater at three colonies (Baccalieu 2.52 µg/g dry wt., Gull 2.53 µg/g and Bon Portage 2.15 µg/g) than at Kent Island in the Bay of Fundy (1.53 µg/g dry wt.). Nevertheless, the geometric mean blood and egg mercury concentrations at each colony were highly correlated (Pearson $R = 0.96$, $p = 0.01$). We will discuss the insights provided by $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ for interpreting differences in blood and egg mercury concentrations among and within colonies. We will also discuss the potential toxicological significance of the observed blood and egg mercury concentrations for Leach's storm-petrels breeding in Atlantic Canada. Leach's storm-petrel can be used as an indicator of methylmercury dynamics in marine mesopelagic food webs.

**194 Mercury exposure and altered parental nesting behavior in a free-living songbird**

*A. Hartman, J.T. Ackerman, M. Herzog, US Geological Survey / Western Ecological Research Center*

Reproduction is among the most sensitive endpoints of methylmercury exposure in birds. At lethal concentrations, methylmercury exposure can impair reproduction through direct mortality of offspring. However, the mechanisms by which sublethal methylmercury concentrations impair avian reproductive success are unclear. We investigated maternal mercury concentrations and maternal incubation behaviors of 59 free-living tree swallows (*Tachycineta bicolor*) in the Central Valley of California. We observed differences in tree swallow incubation behavior and performance with increased maternal mercury concentrations. Females with higher mercury concentrations spent significantly less time on the nest, took more frequent and slightly shorter incubation recesses, and were more likely to take recesses that resulted in substantial nest temperature decreases than females with lower mercury concentrations. As a result, by the end of the typically 14-day incubation period, we predicted that eggs of females with the highest mercury concentrations cumulatively lost almost one day of incubation time compared to the eggs of females with the lowest mercury concentrations. Less time spent incubating and caring for eggs could lengthen the incubation period, reduce egg hatching success, and decrease nest survival. Our results are among the first to demonstrate that methylmercury exposure can alter parental nesting behaviors in a population of free-living songbirds.

**195 Hepatic transcriptome variations in three avian species reflect their susceptibility to mercury**

*N.K. Karouna-Renier, USGS Patuxent Wildlife Research Center; R.S. Cornman, U.S. Geological Survey, Fort Collins Science Center*

Previous studies have confirmed clear differences in the sensitivity of various bird species to methylmercury. These studies have ranked species based on survival (LC50) from laboratory exposures *in ovo* to injected methylmercury (MeHg). Although wide variation in LC50 exists, the causes of these variations in sensitivity are unknown. Organisms have a variety of mechanisms that protect against mercury toxicity. Genetic variations and gene expression patterns related to these processes influence susceptibility and allow less sensitive species to survive, while their more sensitive counterparts succumb. In order to describe the mechanisms and strategies that underlay the differences in sensitivity to methylmercury, we used RNAseq to compare hepatic RNA expression patterns in three bird species that show varying sensitivity to mercury: laughing gull (*Larus atricilla* low sensitivity), American kestrel (*Falco sparverius* – high sensitivity) and osprey (*Pandion haliaetus* – high sensitivity). Eggs were injected with a single dose of MeHg (final concentration = 0.05, 0.1, 0.2, or 0.4 ug/g) to simulate maternal deposition of mercury into the egg. Liver was collected and transcriptome profiles were examined. We found striking differences in the number and identity of differentially expressed genes between the three species at each common dose. For example, at 0.1 ug/g/ MeHg, 4525 differentially expressed genes (DEG; 356≥log2 fold change) relative to oil-only controls were identified in laughing gulls (adjusted p-value < 0.05). Osprey (12 DEG, 11≥log2 fold change) and kestrels (95 DEG, 66 ≥log2 fold change) exhibited much lower, and qualitatively different expression of mRNA, suggesting that laughing gulls are better able to mount a response to Hg than their more sensitive counterparts.

**196 Does anticoagulant rodenticide exposure have lasting effects on sensitivity to subsequent anticoagulant rodenticide exposure in raptors?**

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A seminal question in wildlife toxicology is whether exposure to an environmental contaminant can evoke subtle long-lasting effects on body condition, physiological function and survival. An often-cited study in laboratory rats demonstrated that a single oral dose of the second-generation anticoagulant rodenticide (SGAR) brodifacoum prolongs blood clotting time for just a few days, but weeks later when rats were exposed to the first-generation AR (FGAR) warfarin, coagulopathy was more pronounced in brodifacoum-exposed rats compared to naïve rats exposed to warfarin. This observation was attributed to partial inhibition of hepatic microsomal vitamin K epoxide reductase (VKOR) activity for weeks after exposure (Mosterd & Thijssen 1991). To investigate this phenomenon in non-target birds, studies were undertaken in American kestrels (*Falco sparverius*) to demonstrate brodifacoum toxicity, and subsequently examine effects on clotting time during repeated AR exposures. Kestrels fed diets containing 0.3, 1 or 3 mg brodifacoum/kg ww for 7 days exhibited dose-dependent evidence of bruising, hemorrhage, and coagulopathy. When a 7-day dietary brodifacoum exposure at 0.5 mg/kg diet was terminated, prolonged clotting times were restored to baseline values within a week. Brodifacoum appears to target the same enzyme, VKOR, in American kestrels as in mammals, and has similar inhibition efficiency for VKOR containing hepatic microsomes (IC50 of 0.22 µM in kestrels and 0.15-0.26 µM in mammals). Since the residual brodifacoum concentration in kestrel hepatic microsomes (< 0.02 µM) is much lower than the IC50, we were not able to relate in vitro VKOR activity inhibition to in vivo signs of intoxication in kestrels as reported in the laboratory rat. In order to examine the hazard associated with sequential exposure to multiple ARs, kestrels were initially exposed to an untreated diet or diets containing either the FGAR chlorophacinone (1.5 mg/kg) or the SGAR brodifacoum (0.5 mg/kg) for 7 days. Then, following a 7-day recovery period, all birds were challenged with a low dose of chlorophacinone for 7 days (0.25 mg/kg diet, previously demonstrated to evoke minimal effects on clotting time; Rattner et al. 2015). At these exposure levels, long-lasting effects of AR exposure and residues on clotting time were not apparent. Additional studies using environmentally realistic doses and route of exposure are underway to further test this hypothesis.

**197 Avian interspecific differences in rodenticide sensitivity: Amino acid sequence and mRNA expression ratio of VKORC1 and VKORC1L1**

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Worldwide use of anticoagulant rodenticides (ARs) for vertebrate pest control has frequently led to the unintentional exposures of non-target animals, especially raptors, to these poisons. The median lethal dose (LD50) for anticoagulant rodenticides (ARs) among animals suggest that avian species seem more resistant to ARs than mammals. However, the LD50 for ARs in the American keestrel (Falco sparverius) is lower than that in poultry. Considering both the low values of LD50 in raptors and the frequent poisoning of non-target wild birds, especially raptors, implies raptors have different susceptibility to ARs than poultry. These facts suggest that it is inappropriate to extrapolate the LD50 data from poultry to the risk assessment for raptors. In order to reveal the mechanism of the incidents, this study focused on the avian vitamin K 2, 3-epoxide reductase (VKOR) that is the target protein of ARs. We clarified the interspecific differences in VKOR activity and inhibition related to amino acid sequence and mRNA expression of VKORC1 and VKORC1-like1 (VKORC1L1). VKOR activity of owls, hawks, falcon and surprisingly, canaries, was lower and inhibited by warfarin more easily than that of chickens and turkeys. The amino acid sequence of VKORC1 and VKORC1L1 implied that the value of Ki for VKOR activity to ARs could depend on the amino acid at position 140 in the TYX warfarin-binding motif in VKORC1, and other amino acid mutations in VKORC1L1. The mRNA expression ratio of VKORC1:VKORC1L1 differed between turkey (8:1) and chicken (2:3) liver. Hence, both the Ki of specific VKORC1 and VKORC1L1, and the mRNA expression ratio would cause avian interspecific difference of the VKOR inhibition ratio. Our study also demonstrated the high inhibition ratio of raptor VKOR activities and surprisingly that in canaries as well. These factors are the most likely to contribute to the high sensitivity to ARs found in raptors. Furthermore, our strategy is worth identifying novel avian species such as canaries which could be susceptible to ARs. Our study will help reveal the mechanism of secondary poisonings in raptors by ARs.

**198 Tracking the atmospheric exposure to halogenated flame retardants on the back of a gull**

*M. Sorais, UQAM – TOXEN / Biological Sciences; M.J. Mazerolle, Laval University / Wood and Forest Science; J. Giroux, J. Verreault, University of Quebec at Montreal / Department of Biological Sciences*

A large suite of halogenated flame retardants (HFRs) including polybrominated diphenyl ethers (PBDEs) and certain emerging compounds have been detected in tissues and eggs of ring-billed gulls (*Larus delawarensis*) nesting in the Montreal area (QC, Canada). Elevated plasma concentrations of the highly hydrophobic DecaBDE in male gulls were found to correlate with the time spent in specific foraging habitats such as landfills. Landfills are known hotspots of emissions for airborne HFRs that are efficiently transported in the atmosphere. Hence, gulls feeding in and around landfills can be highly exposed to these chemicals via dust and particle ingestion and inhalation. However, no method has as yet been developed to quantify this type of exposure. Therefore, we recently designed a miniature passive air sampler that can be carried by gulls for the collection of gas- and particle-phased HFRs in air. The present study aimed to: a) assess the spatial associations between the accumulation rates of individual HFRs collected in bird-borne passive air samplers and the different habitats visited by gulls, and b) model the specific contribution of landfill usage in the Montreal area to the accumulation rates of HFRs in the samplers. We equipped 67 nesting gulls with a miniature passive air sampler and a GPS datalogger in order to collect HFRs in air while monitoring their movements during two weeks. The main PBDE congeners detected in the samplers were the major components of the commercial mixtures PentaBDE (BDE-47 and -99) and DecaBDE (BDE-209). Also, a few emerging HFRs (hexabromobenzene, Dechlorane-604 CB and Dechlorane plus) were detected, although at low levels. Important variations in accumulation rates were found between individual samplers for PentaBDE, OctaBDE and DecaBDE congeners. Spatial analysis showed that the accumulation rates of PBDE mixtures were more aggregated than emerging HFRs around punctual local sources, such as landfills. The accumulation rates of all PBDE mixtures and Dec604-CB were indeed found to positively correlate with the proportions of time spent by gulls in landfills. However, such correlations were not observed for the emerging HFRs, which suggests alternative sources for these chemicals. This is the first study to address interindividual variations in atmospheric HFR exposure in birds, while providing valuable information on the local sources of these ubiquitous chemicals in a highly urbanized region.

**199 Extracts of passive samplers deployed in variably contaminated wetlands in the Athabasca oil sands region elicit unique effects in avian hepatocytes**

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Deployment of passive samplers in wetlands within the Athabasca oil sands region represents an effective means to conduct environmental monitoring without requiring the collection of wildlife tissue samples. In this study, graded concentrations of semi-permeable membrane device (SPMD) extracts, deployed at 4 wetlands with variable burdens of polycyclic aromatic compounds (PACs), were administered to chicken and double-crested cormorant (DCCO) embryonic hepatocytes. Effects on 7-ethoxyresorufin-*O*-deethylase (EROD) activity and mRNA expression were determined. PAC concentrations were variable among sites and alkyl-PACs were more abundant in SPMDs than parent compounds. EROD activity and *Cyp1a4* mRNA expression permitted the ranking of wetland sites based on aryl hydrocarbon receptor-mediated endpoints (e.g. EROD $EC_{Thr}$ was lowest and *Cyp1a4* mRNA induction highest in extracts from the wetland with the greatest PAC concentrations). Two avian ToxChip PCR arrays (chicken and DCCO) provided a more exhaustive transcriptomic evaluation across multiple toxicological pathways following exposure to the SPMD extracts. The wetland sites with the greatest PAC concentrations led to the alteration of the most genes on the ToxChip arrays (e.g. 12-15/43 genes on the chicken ToxChip). Exposure of avian hepatocytes to SPMD extracts from variably contaminated wetlands highlighted traditional PAC-related toxicity pathways as well as other novel mechanisms of action. Extrapolation to real world exposure scenarios must consider the bioavailability of the extracted PACs contained in the SPMDs compared to those in exposed organisms; however, such approaches show promise in terms of identifying hotspots of chemical concern in the natural environment.

**200 Polycyclic aromatic hydrocarbon exposure impacts on Sanderling pre-migratory fuelling and migration**

*K. Bianchini, University of Saskatchewan – Toxicology Centre / Toxicology; D. Newstead, Coastal Bend Bays & Estuaries Program; C.A. Morrissey, University of Saskatchewan / Biology*

Long-distance migratory birds can make non-stop flights of thousands of kilometers without access to supplementary food or water. Accomplishing these extreme endurance feats requires that birds rapidly store adequate fuel loads prior to departure. Efficient fuelling is critical for migratory birds because fuel loads and fuelling rates affect reproductive performance and survival during migration. The polycyclic aromatic hydrocarbons (PAHs) found in oil pollution have the potential to interfere with pre-migratory fuelling physiology. However, a link between PAH exposure and impaired pre-migratory fuelling has yet to be established. We used a combination of captive and field techniques to determine if PAH contamination affects pre-migratory fuelling and the subsequent migration of Sanderling (*Calidris alba*), a long-distance migratory shorebird. For captive experiments, we orally exposed 49 wild-caught Sanderling with a commercial PAH mixture over a 21-day period of pre-migratory fuelling. In the field, we measured the mass and plasma metabolite levels of 170 Sanderling at six staging sites in Gulf of Mexico (GOM) and over 375 Sanderling in Chaplin Lake, Saskatchewan. Coded radio telemetry tags were attached to a subset of birds to track migratory movements using the Motus radio-telemetry array. At all staging sites, we measured sediment total PAH concentrations to assess oil contaminant exposure. Captively dosed Sanderling showed reduced pre-migratory mass gains relative to controls, which were associated with reduced serum bile acid and elevated serum lipase and creatine kinase concentrations. Similarly, in the field, plasma metabolite levels revealed that Sanderling in Louisiana, where sediment PAH concentration were the highest, had the lowest fuel deposition rates. Radio telemetry data showed that fuel loads were negatively correlated with stopover durations and departure dates, where leaner birds departed later. Moreover, individuals that departed later from the GOM also showed later arrival and subsequent departure dates in Saskatchewan. Our results thus confirm that PAH exposure can impair avian pre-migratory fuelling. We also show that impaired fuelling can cause delays in migration that carry over from one staging site to the next. This work thus highlights the significant threat of PAH exposure to long-distance migratory birds.

## Translating Environmental Science into Improved Outcomes and Policy

**201 Translational research success stories: The role of industry, academic, and agency collaboration**

*K. O'Reilly, Exponent / Environmental and Earth Science*

From leaking storage tanks at a local service station to large oil spills, the fate of petroleum in the environment interests the public, policy makers, and those in the oil industry. Research on the fate and risks of hydrocarbons has long been conducted by scientists associated with academic institutions, environmental agencies, trade groups, consulting firms, and oil companies. Throughout the modern environmental era, collaboration across these entities has been critical in integrating the results of basic research into regulatory policy and remediation technologies.

Collaboration provides a conduit from the bench through pilot systems and field-testing to real world sites and facilities. This presentation will discuss the role of collaborative translational research from the development of monitored natural attenuation through the growing use of non-targeted chemical analysis to assess risks associated with petroleum biodegradation intermediates in the environment. The presentation will also highlight the application of collaborative research to improve regulatory policy and technical guidance.

**202 Objective-based regulation of offshore oil and gas in Australia**

*T. Reitsema, NOPSEMA*

This presentation provides an introduction to the approach used by Australia's National Offshore Petroleum Safety and Environmental Management Authority (NOPSEMA) in regulating 'planned discharges' (such as produced water or drill cuttings/mud) from the offshore oil and gas industry. NOPSEMA commenced its environment function in 2012, and administers an objective-based form of regulation. This regulatory method varies from the prescriptive regulatory approach, in that responsibility for ensuring protection of the environment lies with those who create the risk. An objective-based regulatory regime sets high level requirements that must be achieved, but does not specifically prescribe how those requirements must be met. Requirements are determined through the assessment process, and are set within accepted environment plans which are legally binding documents. The objective-based regulatory approach offers benefits such as scalability and flexibility relative to the risk profile of the activity, as well as encouraging adoption of best practice management. It also raises challenges such as the application of the environmental risk assessment process to drive acceptable environmental outcomes, and the principle of reduction of impacts and risks to ALARP (as low as reasonably practicable). For example, with previous prescriptive legislative requirements for oil in water content for produced water being removed there is a benefit in ability to drive best practice, with a challenge of needing to ensure that standards that are set are in fact acceptable and ALARP. This presentation will provide information on the regulatory approach used by NOPSEMA, and provide an opportunity for dialogue, also in relation to approaches used in other jurisdictions.

**203 Reform of Environmental Regulations – What's this got to do with environmental toxicology and risk assessment?**

*T.S. Bingman, DuPont Corporate Remediation; R.G. Stahl, E.I. DuPont de Nemours and Company / Corp Remediation; B. Grimsted, Peoneer Technologies Corp; C. Waldron, Pioneer Technologies, Inc.*

A key initiative of the current administration is streamlining environmental regulations in order to increase efficiency and lower regulatory impacts on American industry. In early 2017, Executive Orders 13771 and 13777 were issued outlining steps in the regulatory reform process. Such efforts are not new. In 1978, President Carter set out to reduce regulations, and similar efforts have been undertaken by every president since. EO 12866, signed by President Clinton in 1993, required that risk assessment be used to help evaluate the cost of new regulations, and if implemented whether they would improve the health and welfare of the American public. In this presentation, we discuss how these regulatory reform efforts have shaped today's use of risk-based decision-making for the management of environmental and human health policies and practices in the US.

**204 Cumulative risk analysis of contaminant mixtures in tap water: California case study**

*T.L. Stoiber, University of California-Davis; A. Temkin, S. Lunder, D. Andrews, O.V. Naidenko, Environmental Working Group*

Tap water in many communities in California has multiple contaminants at levels that meet national drinking water regulations, but are far from meeting public health goals for what scientists consider safe. National drinking water contaminant standards set under the Safe Drinking Water Act are a compromise between health benchmarks that define a minimal risk level on one side, and technical feasibility and cost of removal on the other side. Existing national and state regulations assess both drinking water standards and health benchmarks one chemical at a time, even though tap water nearly always contains a mix of contaminants. Systems with higher risk are often those that serve smaller, rural populations and drought conditions in California have exacerbated problems with common drinking water contaminants including disinfection byproducts and arsenic. Using California as a case study, we developed a cumulative risk approach for tap water contaminants conceptually similar to the other cumulative approaches developed for air pollution. We analyzed drinking water contaminant mixtures in tap water using the California state drinking water dataset for 2015 for most contaminants and the USEPA's Third Unregulated Contaminant Monitoring Rule (UCMR3) dataset for 2013-2015. Cumulative cancer risk estimates were derived by summing the cancer risk estimates for individual contaminants in tap water provided by individual water utilities. Contaminant co-occurrence analysis evaluated the simultaneous presence of contaminants with cancer and non-cancer health benchmarks. Based on the cancer potency and relative occurrence, cancer risk from tap water contaminants in California was primarily driven by the presence of arsenic, hexavalent chromium, and disinfection byproducts. Approximately 90 percent of the water systems had at least one contaminant at levels above health-based guidelines and approximately 63 percent had at least two contaminants above. Almost a quarter of the systems had four or more contaminants. Cumulative water contaminant analysis that accounts for the adverse health effects of co-occurring contaminants can identify water systems and communities most in need of resources for safe drinking water. Additionally, the health and economic benefits of reducing the levels of multiple contaminants with specific water treatment technologies should be considered when setting regulatory limits.

**205 Product Design as Institutional Work for Continuity and Change in Fields: Insights from a Study of Toxicogenomics for Ecological Risk Assessment**

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Much has been written about how toxicogenomics, i.e. collection and interpretation of information about gene and protein activity in cells in response to toxic substances, has the potential to make the assessment of chemical risks to health and environment cheaper, faster and more ethical as regards animal welfare. In part 1 of this paper, an analysis of the discourse of toxicogenomics reveals a fundamental tension: it is simultaneously constructed as just another tool in the risk assessment toolkit and as a revolutionary change heralding a transformative paradigm shift in toxicology. We compare the "tool" and "revolution" perspectives and identify key aspects of toxicogenomics where they diverge and this tension is most acute: envisioned uses; perceived barriers to adoption; interpretation of 'omics data; and approaches to validation. How do advocates of toxicogenomics navigate this tension? In part 2 of this paper, we address this question through a case study of a team of scientists (hereafter 'the product design team') undertaking the development of new quantitative PCR arrays (EcoToxChips) for characterization, prioritization, and management of environmental chemicals of concern. Because chemical risk assessment is an institutionalized field of professional practice, with methods and routines widely accepted and taken for granted, we examine changes in practices due to toxicogenomics through the lens of "institutional work," which is action taken to create, maintain and disrupt institutions. Drawing on qualitative data (scientific and regulatory texts; interviews and focus groups; participant observation of the product design team), we analyze the activities of the product design team, including interactions with regulators and other potential users of the EcoToxChip, as different forms of institutional work. By documenting key elements of prevailing, institutionalized practices and tracking their fate through the product design process, we show how some are maintained and reproduced, others are disrupted and abandoned, while new institutional elements are introduced by the EcoToxChip. We illustrate how, through the product design process, academic researchers' abstract, longer term "vision" of a transformative paradigm revolution is brought together and reconciled with regulators' concrete, shorter term "practical needs" for specific tools such that the new product affords both institutional continuity and change.

### 206 A Comprehensive Dispersant Hazard Assessment Based on Nearly 50-Years of Aquatic Toxicity Data

*A.C. Bejarano, Research Planning Inc. / Department of Environmental Health Sciences*

The use of dispersants as an oil spill response tool often causes concerns among the public and the scientific community because of their potential toxicity to aquatic organisms. The aquatic toxicity of dispersants has been extensively investigated under controlled laboratory exposures providing answers to scientific questions, while generating information applicable to a real-world issue and useful in addressing public concerns. Thus, mining these data would facilitate a boarder understanding of their toxicity, and enable a more comprehensive hazard assessment. Data from multiple studies were evaluated for their quality based on criteria allowing assessments of reliability. Although in recent years there has been an emphasis on the need of standardized aquatic toxicity testing, nearly a quarter of sources did not provide sufficient information on which to assess their quality. Between 1969 and 2018, over 100 aquatic species have been used in dispersant-only toxicity testing, with lethality (LC50) as the most commonly reported endpoint. The most extensively tested dispersants have been Corexit 9500 and Corexit 9527. The application of most of these data to real-world conditions is constrained by laboratory test conditions: data are primarily from constant static/static renewal tests under standard test durations (e.g., 96 h), which are not presentative of exposures under field conditions. However, data were used to develop dispersant-specific species sensitivity distributions (SSDs) and hazard concentrations protective of 95% of the species assemblage (HC5). Most of the 91 SSDs for 54 dispersants had HC5s falling within the moderate to slightly toxicity range. Data interpretation within the context of concentrations based on operational application rates indicated that HC5 exceedances may occur in the top few meters of the water column. However, under more realistic conditions, dispersant exposures would be short with concentrations rapidly declining below toxic levels. The goals of this presentation are to: 1) provide an overview of the body of scientific knowledge generated over several decades on the toxicity of dispersants; 2) offer recommendations on improved testing and reporting; 3) highlight possible research that is needed to translate data from standard tests into information that could inform decision making.

### 207 An avian reproduction study historical control database: A tool for data interpretation

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Avian reproduction studies are a regulatory requirement for pesticides in many regions. The data often require careful interpretation due to the nature of the study design. Here we present the historical control dataset for bobwhite quail and mallard duck reproduction studies performed at the Evans Analytical Group LLC avian toxicology laboratory over the period 1985 – 2016. The analysis demonstrates the stability of reproductive parameters over time and good agreement to normal control ranges as required by the regulatory test guidelines. The major source of variation is shown to be within study variation. We believe the analysis and evaluation presented here can facilitate the development of practical guidance that can be implemented in regulatory programmes requiring this test. Data from 301 bobwhite quail and 292 mallard duck studies conducted between 1984 and May 2016 were colalted into an Excel spreadsheet. For mallard duck all birds were obtained from a single supplier or hatched at the laboratory from parents obtained from the same supplier. For bobwhite quail a total of 18 suppliers have been used. The data indicate that control response for both species has been stable over time (30+ years). Significantly, the means are in good agreement with the OECD guideline for normal (typical) values and the OSCPP guideline control responses for 'satisfactory' tests. The variation within a study (biological and experimental variation between replicates or pens) accounts for most of the variation of the historical control datasets for the different reproductive endpoints i.e. 64.9 – 93.4%. Between studies variation only explained a relatively small percentage of the total variance (5.7 – 35.1%). In the case of bobwhite quail, where the contribution to the total variance of the bird source (supplier) could be calculated, this factor accounted for an even smaller proportion (0.3 – 12.9%). These results indicate that the majority of the variation is from the intrinsic biological variability of the reproductive parameters and typical experimental variation. The power analyses show that greater replication would not dramatically increase the power properties for most endpoints. The HCD approach can therefore be useful to aid interpretation, improve regulatory decision making and minimise the need for repeat testing.

### 208 Actual and Perceived Risks in the Development of Water Management Strategies for Rural and Indigenous Communities

*M. Serville, Trent University / Environmental and Life Sciences; C.D. Metcalfe, Trent University / Water Quality Centre*

Members of rural and Indigenous communities may have concerns related to potential exposure to microbiological and chemical contaminants in their sources of drinking water. In order to develop a water management strategy that addresses these community concerns, it is important to understand the perceptions of risk among community members who are directly involved in the management of the water resources and communication of risks to the public. The perceptions of these professionals can influence the types or levels of risks they will tolerate, the compromises they make when implementing standards, and the strategies they put in place to manage and communicate risks. Further, multiple perceptions must be acknowledged and understood so that the water management sector can accommodate these points of view. In this study, a mixed-methods convergent parallel approach was used to assess actual risks from microbial and chemical contamination of drinking water for two rural communities located in mixed-use watersheds in Canada and the Caribbean, respectively. Actual risks were determined from quantitative data on the levels and sources of fecal bacteria, and concentrations of current use pesticides (CUPs) and chemical indicators of wastewater contamination in source water and treated drinking water using a hazard quotient (HQ) and hazard index (HI) approach. Perceived risks will be determined from qualitative data collected by interviewing water managers and health professionals within the communities. Quantitative and qualitative data will be integrated for an in-depth interpretation of how data on both actual and perceived risks can be applied to the development of water management strategies that foster effective risk communication, gain the trust of consumers and motivate consumers to respond positively. We anticipate that by translating scientific research into policy interventions, effective water management strategies can be developed.

# Linking Mechanism of Action to Physiological and Ecological Effects in Aquatic Species

### 209 Window of sensitivity to chemically induced alterations in gonad histology and reproductive function in largemouth bass (*Micropterus salmoides*)

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Field studies have shown a high prevalence of intersex in smallmouth bass (*Micropterus dolomieu*), and to a lesser extent largemouth bass (LMB; *Micropterus salmoides*), populations in the Chesapeake Bay watershed. The intersex phenotype has been correlated with the presence of the agricultural pesticide atrazine, which is widely used and one of the most commonly detected pesticides in the watershed. However, it is not known whether this intersex phenotype is associated with reduced reproductive function, which could lead to possible impacts at the population level. Adult LMB have been shown to be sensitive to induction of intersex in response to chronic exposure to a model estrogen (17alpha-ethinylestradiol; EE2) over a complete reproductive cycle. Late summer to fall is the period of early gonad recrudescence when spermatogenesis and oogenesis are beginning in preparation for the spawning season in the spring. Our objective was to assess whether early gonad recrudescence was a period of sensitivity for induction of intersex and/or alterations in reproductive function. Adult LMB were exposed in outdoor pond mesocosms from post-spawning through early gonad recrudescence to either EE2 (2.4 ng/L), or a mixture of endocrine-active substances commonly detected in the Chesapeake Bay watershed, atrazine (5.4 µg/L) and estrone (47.9 ng/L). Samples were collected from fish just prior to exposure (July), at the end of the exposure period (December), just prior to spawning (April), and post spawning (May). Endpoints at various levels of biological organization were evaluated, including: gene expression, plasma hormone concentrations, gonad stage, gonadosomatic index (GSI), liver somatic index (LSI), presence of testicular oocytes, sperm motility, number and volume of spawns, and 24 h embryo viability. Results indicated significantly lower GSI in pre-spawn females and males of the EE2 treatment and lower GSI in the mixture treatment females compared to the control. There was also a trend of lower sperm motility and egg volume in these treatments indicating potential reduced reproductive function. Of the individuals from the EE2 treatment that were externally identified as females just prior to spawning, two contained testes and one contained a testicular lobe and an ovarian lobe when examined at necropsy. This study presents evidence that early gonad recrudescence is a window of sensitivity for induction of intersex and potential reduction in reproductive function.

### 210 Is elevated organohalogen contaminant exposure linked to thyroid- and steroid-related gene responses in St. Lawrence beluga whales?

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Elevated concentrations of polychlorinated biphenyls (PCBs), chlorinated pesticides and halogenated flame retardants (polybrominated diphenyl ethers (PBDEs) and emerging compounds) have been reported in blubber of beluga (*Delphinapterus leucas*) from the St. Lawrence Estuary (Canada). It has been postulated that exposure to these contaminants might play a role in the non-recovery of this endangered population. In vitro and in vivo studies of mammals have shown that PCBs, PBDEs and chlorinated pesticides can perturb the homeostasis of circulating thyroid and steroid hormones or alter the expression of genes involved in their regulation. However, no study has yet investigated the linkages between concentrations of any contaminants in this vulnerable beluga population and impacts on endocrine axes. A suite of organohalogens (PCBs, chlorinated pesticides, PBDEs, and emerging flame retardants) was measured in blubber biopsy samples from 45 free-ranging male belugas from the St. Lawrence Estuary. Contaminant concentrations were related to the expression of gene transcripts measured in skin biopsy samples, and coding for nuclear receptors and proteins involved in the regulation of thyroid (*Dio1*, *Dio2*, *Thrα*, *Thrβ*) and steroid hormones (*Esrα*, *Hsd11β2*, *Hsd17β2*, *Nr3c1*, *Star*) as well as the metabolism of xenobiotics (*Ahr*). The four most abundant organohalogens in beluga blubber were in decreasing order $\Sigma_{37}$PCB, *p,p'*-DDE, $\Sigma_{29}$PBDE, and *trans*-nonachlor. $\Sigma_{37}$PCB, *p,p'*-DDE, *trans*-nonachlor and HCB concentrations were positively correlated with the transcription of *Dio2* and *Esrα*. Hexabromobenzene concentrations positively correlated with three steroid-related genes *Esrα*, *Hsd11β2* and *Nr3c1* as well as *Ahr*, while Dec-604 CB concentrations were negatively associated with the transcription of glucocorticoid and thyroid genes (*Hsd11β2*, *Nr3c1* and *Thrβ*). Results suggest that several biological functions including growth, development, reproduction, and energetic metabolism may represent potential targets for organohalogen exposure-related effects in this population. A suite of metabolites including amino acids, biogenic amines and fatty acids is currently being analyzed in skin of these belugas using metabolomics. Relationships between these markers, gene transcripts and levels of organohalogen compounds will be examined. Present study will contribute to better understand the health risks associated with elevated organohalogen exposure in endangered St. Lawrence Estuary belugas.

### 211 Connecting Developmental Thyroid Disruption to Impaired Reproductive Success in Fathead Minnows

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Thyroid disrupting compounds (TDCs) are known to interfere with normal thyroid hormone (TH) signaling. During embryonic and juvenile development, thyroid hormones modulate a variety of biological processes such as neurogenesis and the growth of the skeletal and muscular systems. Therefore, the majority of research on early life-stage (ELS) thyroid disruption has focused on its effects on growth and development. However, recent research has shown that ELS TDC exposure can also have adverse effects on reproduction later in life. Specifically, fathead minnows exposed to propylthiouracil (PTU), an anti-thyroid drug known to inhibit the synthesis of thyroxine (T4), during early development (from hatch through 42 days post hatch) experienced a 50% reduction in fecundity relative to controls. Interestingly, this statistically significant reduction in fecundity occurred when males, but not females, were subjected to ELS PTU exposures. After ruling out the possibility that ELS thyroid disruption altered testicular function, it was hypothesized that the observed reductions in fecundity resulted from changes in male reproductive behavior. To investigate the potential for and mechanism underlying PTU-induced alterations in male behavior, brains of PTU-exposed and control males were collected immediately after exposure for transcriptomic analysis. Of the genes that were found to be differentially expressed between the brains of PTU-exposed and control males, several were associated with sex steroid signaling. Specifically, PTU-exposed males experienced significant reductions in the expression of aromatase, estrogen receptor alpha, and androgen receptor, relative to controls. Given the known role of these genes in sexual differentiation of the male brain, these results provide evidence supporting the hypothesis that ELS chemically-induced hypothyroidism leads to altered brain development and subsequent alterations in behavior. Overall, the results of this study may help link transcriptomic alterations in the brain to alterations in reproductive behavior, which has important population-level consequences.

**212 Non-lethal sampling of the caudal fin and transcriptomics analyses enable marine diesel oil spill effects determination on Pacific salmon**

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Various types of crude and refined oils are involved in marine coastal spills and monitoring their impacts and the effectiveness of cleanup methods remains a challenge due to their highly complex and variable nature. Dispersion of soluble polycyclic aromatic hydrocarbons (PAHs) is influenced by water salinity and relatively little is known about their toxicity at typical salinities experienced in the Pacific Ocean (28-30 ppt). We investigated the PAH composition dispersed after a simulated marine low sulfur marine diesel (LSMD) spill and the utility of a non-invasive method of monitoring the impact on juvenile Coho salmon following a 96 hour exposure to low, medium, and high concentrations (100-1000 mg/L) of LSMD seawater accommodated fractions (LSMD-seaWAF). The sum of 50 known PAHs and alkylated-PAHs (TPAH-50) was measured using gas chromatography/triple quadrupole mass spectrometry daily. In contrast to lethal liver sampling for biological effects determination, the caudal fin is a readily accessible tissue which can be non-lethally sampled with minimal distress to the animal. Building on previous work, we investigated if the caudal fin transcriptome is an effective alternative to the liver to reflect the biological response of Coho salmon to LSMD-seaWAF. Our findings indicate that all LSMD-seaWAF preparations were saturated at ~30 ppb TPAH-50 and that the caudal fin *cyp1a1* gene transcript is a robust, non-sex-biased bioindicator of oil exposure exhibiting a 10-18-fold change in abundance from seawater control animals. RNA-seq analyses to more fully characterize the caudal fin response and identify additional impacted pathways is currently underway.

**213 Developmental and behavioral consequences of cannabinoid exposure in zebrafish**

*K.L. Willett, Z. Miller, D. Carty, J. Gledhill, C.E. Thornton, University of Mississippi / BioMolecular Sciences*

Due to relaxed prohibitions on cannabis use and therapeutic potential of cannabis constituents in the treatment of early-onset pharmaco-resistant epilepsy, exposure of children to these chemicals is increasing. Yet the consequences of developmental exposure to cannabinoids on the potential etiology of subsequent adult or multigenerational toxicity is currently unknown. The goal of this project is to study the toxicity of cannabidiol (CBD) and Δ9-tetrahydrocannabinol (THC) using zebrafish. Our central hypothesis is that developmental exposure to THC and/or CBD changes gene expression in critical genes during development that are, in turn, related to the origins of adult disease. To answer this question, F0 zebrafish were exposed via water from 6-96 hours post-fertilization (hpf) with either THC (0.08, 0.4, and 2 μM), CBD (0.02, 0.1, and 0.5 μM), or control (0.05% DMSO). RNA-seq analysis from 10 and 96 hpf fish revealed several genes that were differentially expressed compared to controls including metabolism (e.g. cyp1a, gstp1, nqo1) and solute carrier family genes (slc26a3.2 and slc51a). In addition to gene expression, larvae were also screened for abnormal development and behavior. Larvae exposed to CBD developed curved body axes, pericardial and yolk sac edemas, deflated swim bladders, and abnormal behavior. THC exposed larvae developed similar abnormalities, but at much higher concentrations than CBD. In order to assess reproductive fitness and latent adult disease, larvae were exposed to sub-LOAEL concentrations of CBD and THC for 96 hours then reared in untreated water. In F0s exposed to CBD and THC, fecundity was significantly decreased, but neither fertilization success nor F1 or F2 larval survival was adversely affected. An adult F0 open field behavioral test indicated a dose-dependent trend of increasing freezing duration after developmental exposure to CBD or THC which supports our hypothesis for cannabinoid related developmental neurotoxicity. This work highlights the need to consider long-term ramifications of early-life exposure to cannabinoids. Supported by the NIH NIDA (R21DA044473-01) and NIGMS (P20GM104932) and the UMMC Molecular and Genomics Core Facility.

**214 Contaminants of Emerging Concern in the Great Lakes: Effects from Simple Exposures to Complex Mixtures**

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Contaminants of emerging concern (CEC), including pharmaceuticals, personal care products and industrial agents affect the health of aquatic organisms while there is a little information known about their inclusive harm to the ecosystem. Previous in vitro studies have documented that concentrated urban-sourced CEC mixtures hyper-stimulated fathead minnow ESR1 beyond the maximum induction of E2. Purpose of the current study was to form a bridge between long term, multigenerational studies conducted on complex mixtures of CECs, and understand the change on molecular and apical endpoints as the complexity of exposure increases. We tested the hypothesis that as the complexity of CECs in exposures increase, the molecular and apical endpoints observed will differentiate from simple exposure endpoints. We assessed the potential of 21 commonly detected CECs on two life stages of fathead minnows: juvenile (survival, escape performance, feeding efficiency, qPCR) and adult (survival, secondary sex characteristics, nest defense, courtship, boldness, plasma vitellogenin concentration, qPCR) after 96-hour flow-through exposures. In addition, we began the process of building a series of complex mixtures to study the CEC effects by using neural network methodology. Interestingly, exposure to ibuprofen showed an increase in body length at the medium concentration (environmental concentration) on larval fathead minnow ($p < 0.05$, ANOVA). We also observed indication of endocrine disruption on our mixture exposure which includes all studied chemicals. We expect that these evaluations will lead us to improve adverse outcome pathway concept by testing same chemical effects at different life stages of fathead minnows, and forming a linkage between behavioral responses and adverse outcomes.

**215 An AOP analysis of selective serotonin reuptake inhibitors (SSRIs) in fish with an emphasis on the cardiovascular and respiratory systems**

*M. McDonald, RSMAS, University of Miami / Marine Biology and Ecology; M.H. Amador, RSMAS, University of Miami*

Selective serotonin reuptake inhibitor (SSRI) antidepressants, such as fluoxetine, are found in measurable quantities in surface and ground waters. Because of their importance in human health, the pharmacokinetics and physiological impacts of SSRI treatment have been well-studied in small mammals and humans. There is also evolutionary conservation of the serotonergic system across vertebrates that, combined, allows for the read-across of known SSRI effects in mammals to potential SSRI impacts on aquatic organisms. Using an Adverse Outcome Pathway (AOP) framework, this presentation will examine the similarities and differences between the mammalian and teleost fish SSRI target, the serotonin transporter (SERT; SLC6A4), and the downstream impacts of elevated extracellular serotonin (5-HT; 5-hydroxytryptamine) – the consequence of SERT inhibition – on cardiovascular and respiratory organ function and on the response to low environmental oxygen levels (hypoxia) of teleost fish. Our data shows that fluoxetine treatment results in a decreased capacity to respond to hypoxia both on the cardiovascular level and on the level of metabolism and could suggest that waterborne fluoxetine exposure, especially when in combination with other SSRIs, may have a negative impact on fish survival when faced with the additional challenge of environmental hypoxia.

**216 Extension of the Human-Relevant Potency-Threshold approach to ecological species for hazard identification, AOPs, and cumulative risk assessment.**

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The European Union has recently adopted Criteria and Guidelines for the identification of endocrine disrupting chemicals (EDCs), defined as chemicals that produce adverse effects via endocrine mechanisms. Identifying and characterizing toxic hazards based on the manner by which adverse effects are produced rather than on the nature of those adverse effects departs from traditional practice and requires a proper interpretation of the evidence regarding the chemical's ability to produce physiological effect(s) via a specific MoA. The ability of any chemical to produce a physiological effect depends on its pharmacokinetics and the potency by which it acts via the various MoAs that can lead to the particular effect. A chemical's potency for a specific MoA – its mechanistic potency – is determined by two properties: 1) its affinity for the functional components that comprise the MoA, i.e., its specific receptors, enzymes, transporters, transcriptional elements, etc., and 2) it's ability to alter the functional state of those components (activity). Recently an empirical approach was developed for determining the minimum level of mechanistic potency necessary for a chemical to be able to act via a particular MoA in humans, called the Human-Relevant Potency-Threshold (HRPT). Its application was illustrated through the development of an HRPT for the estrogen receptor alpha (ERα) agonist pathway of 1E-04 relative to the potency of the endogenous estrogenic hormone 17β-estradiol. The HRPT is useful for distinguishing between chemicals that may be capable of, versus those likely to be incapable of, producing adverse effects in humans via the specified MoA. This approach provides a practical means for addressing Hazard Identification according to the draft criteria for identification of EDCs recently proposed by the European Commission. Here, we show how the HRPT concept can be extended to analogous mechanistic thresholds in fish using comparative data for human and fish estrogen and androgen receptor systems. In addition, a practical method is proposed for using taxa-based potency thresholds for the identification of ecological hazards, as criteria for linking molecular initiating events to downstream key events in Adverse Outcome Pathways (AOPs), and for assigning chemicals to common assessment groups for mixtures risk assessment.

## Fate and Effects of Metals: Biogeochemical Perspective

**217 Understanding the role of DOM on mercury bioavailability – a prerequisite for the narrowing the gap in lab-to-field extrapolation**

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Mercury biogeochemical cycle is strongly affected by the human activities, resulting in a significant increase of Hg compounds concentrations in the environment. What is more, Hg compounds bioconcentrate in biota, biomagnify along the food web and ultimately represent serious concern for the environmental and human health. However, mercury interaction with phytoplankton, central for its incorporation in the food webs, and in particular the role of dissolved organic matter (DOM) as bioavailability modifier is still to elucidate. The objective of this work is to get new insight in the role of the DOM on Hg bioavailability to phytoplankton. To this end, *C. reinhardtii* was exposed to Hg added to natural water rich in DOM from Onega Lake, Russia. Water was sampled from five sites representing the DOC gradient from River Shuya to open lake in March and June 2017. The water was spiked with 1 and 10nM of $Hg(NO_3)_2$ and let to equilibrate 1 or 24h, than the adsorbed and intracellular mercury concentrations in algae were determined after 2h exposure using direct mercury analyzer on freeze-dried algal pellets. Concentrations of Hg in the exposure media were measured with the MERX Automated Total Mercury Analytical System. In paralel the composition and the molar mass of the DOM was characterized by LC-OCD and flow-field flow fractionation – multidetection system. The amount of the SH- reactive groups was quantified too. Chemical speciation of Hg in the absence or presence of DOM was modelled. The results showed that adsorbed and intracellular Hg concentrations decreased as compared with exposure in the absence of DOM. No specific trends in the Hg uptake by *C. reinhardtii* were observed over DOC concentration gradients after 1h or 24h equilibration. However a clear decrease of the Hg bioavailability proportional to the aboundance of the reduced -SH groups was found. The principle component analysis showed that in addition to the abondance of the -SH groups of DOM, the other factors such as the presence and concentration of different nutrients, as well as mercury binding to the Al, Mn and Fe colloids has to be taken into account to understand Hg bioavailability to phytoplankton. The implications of the obtained results are discussed further with respect to the prediction of the mercury incorporation at the base of the food-webs and the narrowing the gap in lab-to-field extrapolation.

**218 Microbial activities versus mercury species reactivity in the deep sea sediment of the Capbreton Submarine Canyon (Biscay Bay, SW France)**

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Due to its high affinity with particulate matter, mercury is readily scavenged from the water column and deposited to bottom sediment particularly in coastal and marine environments causing mercury contamination. In the sediment and under anaerobic conditions, mercury can be converted into monomethylmercury (MMHg), the most harmful form. The net production of MMHg results from both methylation and demethylation reactions mediated by both biotic and abiotic processes. In this work, relation between mercury levels (iHg and MMHg by ID-GC-ICP-MS) and microorganism taxonomic diversity (DNA 16s sequence by MiSEQ) were studied in deep sea surface sediment from the canyon of Capbreton in the Bay of Biscay. Twenty-four stations were investigated within the first 30 km of the canyon area in July 2017. Biogeochemical parameters (carbon content, grain–size distribution) were determined to characterize these samples. In parallel, study of mercury species transformations, from three stations selected according the distance to the coast, were performed in slurry incubations using mercury isotopic tracers under biotic and abiotic conditions. Mercury speciation analyses exhibited a wide range of IHg and MMHg levels from 18 to 972 and 0.150 to 2.11 $ng.g^{-1}$ dw, respectively. IHg and MMHg concentrations increased with depth and distance from the coast, whereas proportion of MMHg decreased along these gradients. Net methylation potentials were only observed under biotic conditions due to the involvement of prokaryotes in the transformation of Hg species. The determination of the taxonomic diversity and the sequencing of *hgcA* gene, a proxy for biotic methylation, have demonstrated the presence of two main families among the Deltaproteobacteria: *Desulfobulbaceae* and *Desulfobacteraceae*. These results reveal the involvement of these methylating strains in the MMHg production. Higher methylation potential was observed for the coastal stations whereas demethylation potential was similar for all stations, suggesting that environmental parameters (e.g. input of continental organic matter) also control methylating microbial activities. This work confirms the important role of the canyon to trap and transfer mercury via suspended matter from the continent to the abyssal plain and to be a reservoir for Hg and reactor for MMHg production mediated by anaerobic bacteria.

**219 Kinetics of Methylmercury Production in Contaminated Sediments**

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Mercury (Hg) is ubiquitous in the environment, occurring naturally and as an anthropogenic pollutant. In anaerobic environments, Hg can be transformed to methylmercury (MeHg), a neurotoxin that biomagnifies in the aquatic foodweb. The transformation of Hg to MeHg is a microbial process, mediated by a diverse group of anaerobic microorganisms. MeHg can also be demethylated, either through biotic processes or through abiotic reactions. The overall MeHg production in an ecosystem is the net balance of microbially-mediated Hg-methylation and both biotic and abiotic demethylation. Often researchers provide estimates of MeHg production levels in natural systems based on methylation and demethylation rates determined from laboratory experiments. Our previous work demonstrated MeHg production by periphyton biofilms harvested from East Fork Poplar Creek in Oak Ridge, TN. In these experiments, the data exhibited kinetics that were inconsistent with the first-order rate expressions typically used to analyze MeHg production data. We hypothesized that apparent non-first order kinetics in MeHg production were the result of competing kinetic reactions that reversibly converted Hg and MeHg to unavailable states. We used measurements of filter-passing Hg and MeHg, and a multisite adsorption model, to show that kinetic sorption reactions can lead to time-varying availability and non-first order kinetics in MeHg production. We constructed a new model to incorporate multi-site kinetic sorption. This model more accurately fit the range of behaviors seen in time course data for methylation and demethylation assays and resulted in much higher estimates of MeHg production than those predicted by single-rate, first-order models. Our current work applies this kinetic model to metabolically active transient storage zones within sediments from East Fork Poplar Creek. Experiments are underway to determine Hg and MeHg sorption and methylation/demethylation rates in the sediments. Sediments from different regions of the creek will be compared, and MeHg production rates will be correlated with measures of geochemistry and sediment microbial activity.

**220 Microbial generation of volatile mercury from dissolved methylmercury in seawater**

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Elemental mercury ($Hg^0$) formation, followed by subsequent evasion from surface seawater, plays a principal role in the marine mercury cycle. However, the relative importance of microbial-mediated conversion of $Hg^0$ from Hg(II) or monomethylmercury (MeHg) in seawater remains unclear. A rapid radioassay method using the gamma-emitting $^{203}Hg$ as a tracer was developed to evaluate $Hg^0$ production by natural coastal bacterial assemblages for Hg concentrations in the low pM range. Bacterioplankton in Atlantic surface seawater collected 8 km off Long Island, New York, and in Long Island Sound water were found to produce $Hg^0$ from seawater containing Hg(II) and MeHg. $Hg^0$ gas evasion rate constants were independent of dissolved Hg(II) and MeHg concentrations and were directly related to bacterial biomass. About 32% of Hg(II) and 19% of MeHg were converted to $Hg^0$ in 4 d in Atlantic surface seawater containing low bacterial biomass, representative of coastal waters. In Long Island Sound water with higher bacterial biomass, 54% of Hg(II) and 8% of MeHg were transformed to $Hg^0$, inferring that bacterial community structures are important determinants for $Hg^0$ formation. Because low temperatures inhibited Hg(II) reduction substantially and bacterial mercuric reductase was detected in our samples, it can be further inferred that bacterial metabolic activities and enzymatic reactions govern $Hg^0$ formation. Decreasing temperatures from 24°C to 4°C reduced $Hg^0$ production rates cell$^{-1}$ from Hg(II) 3.3 times as much as from a MeHg source. Overall, these findings demonstrate the importance of $Hg^0$ formation mediated by naturally occurring marine bacterioplankton.

**221 Bioaccumulation and Translocation Efficiency of Metals in *Spinacea oleraceae* and *Celosia argente*a Grown on Olusosun Dumpsite Soil, Lagos Nigeria**

*O. Olojuola, A. Ogunyebi, University of Lagos, Akoka / Department of Cell Biology and Genetics; K.O. Omoyajowo, University of Lagos, Akoka / Cell Biology and Genetics / Environmental Biology*

The primary aim of this study is to determine the bioaccumulation factor (BAF) and translocation factor (TF) of heavy metals in two vegetables (*Spinacea oleraceae* and *Celosia argentea*) grown on soils collected from Olusosun dumpsite and that of University of Lagos biological garden (control). Planting of these two plant species was achieved within 4 weeks and harvested following standard analytical procedures. Result shows that metallic concentrations were significantly higher in both species of vegetable samples grown on Olusosun dumpsite soil in comparison to the control. Values for Zn, Cu and Ni were within FAO/WHO recommended level except for Pb (2.20-3.00 mg/kg) and Cr (0.62-1.98 mg/kg) in Olusosun dumpsite that had values above the FAO/WHO recommended limit of 2.0 mg/kg and 0.3 mg/kg respectively. In both vegetable species grown on soil samples collected from both sites (A and B) of Olusosun dumpsite, BAF values for Zn (0.124-0.154), Cu (0.07-0.096), Cr (0.025-0.034), Pb (0.023-0.029) and Ni (0.001-0.036) were less than one (1) showing that these plants only absorb heavy metals but did not accumulate them. Translocation factor (TF) was lesser than 1 in both plant species, and thus follow an order of Zn>Pb>Ni>Cu>Cr in *Celosia argentia* and Zn>Cu>Cr>Pb>Ni for *Spinacea oleracea*. Based on Sutherland classification, site A of Olusosun dumpsite soil was in moderate enrichment category for Zn (2.31), Cu (3.95), Pb (2.50) while site B of Olusosun dumpsite soil was in minimal enrichment category for Zn (1.50), Cu (1.29) and Pb (1.50). The study concluded that the use of dumpsite soil for growing vegetables has increased the concentration of heavy metals (Pb, Cu, Zn, Ni, Cd, and Cr) in their different parts and may pose a potential threat to human health in the long-term term.

**222 Metals removal from water for hazard classification**

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Metals usually enter aquatic ecosystems in an oxic environment and associated with particles. It is important to understand this initial fate process in regards to partitioning, speciation and resulting biological effects. In addition, current European Union regulations and the global GHS system mandate a hazard evaluation, which includes the assessment of Rapid Degradation (greater than 70% within 28 days).. A research program investigated the support for metal removal, partitioning, and transformation as a surrogate for rapid degradation for metals, through the examination of laboratory, field, and modeling data. A series of laboratory and modeling evaluations were conducted to address the following questions: Are metals, such as copper (Cu) and nickel (Ni), removed from the water column of freshwater systems and if so, what is the rate of removal? Can existing standard methods, like Transformation/Dissolution OECD 29 and OECD 308 be used or extended to measure metal removal? How do various test method conditions affect metal removal? What sediment substrates characteristics affect metal removal? Multiple dried vs. non-dried sediments were tested in batch reactors for 96 h and 28 d. Buffalo River, a standardized substrate , typically removed 70% Cu and Ni from the water column at 1 mg/L loading. Incubated sediments removed metals significantly faster than non-incubated sediments ($p < 0.03$). Higher sediment loading rates removed metals faster as expected. Sediment type and loading rates affected pH, which started at 6.0. Cu removal (96 h) and resuspension (1 h post 96 h) resulted in no significant increase in Cu. Removal rates of various metals could be reasonably predicted with modeling based on mechanistic processes. Results show 70% of Ni and Cu are removed from the water column using this OECD 29 – Extension test method, using a variety of sediments and conditions. These data can be used in a weight of evidence to demonstrate that metal rapid removal from the water column is occurring in natural systems.

**223 Metal removal from the water column for chronic hazard classification: Method development for an extended transformation/ dissolution protocol**

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The UN GHS and the EU Classification, Labelling and Packaging (CLP) hazard classification schemes include the concept of degradation whereby rapid degradation from the water column results in different classification cut-off values and categories. For metals and metal compounds, the assessment of environmental transformation may be taken into account. It was evaluated if an extension of the transformation/dissolution protocol (T/DP) could be used for assessing the rapid removal of metals from the water column. To this end, the T/DP method was extended with 2 parts: T/DP-E Part 1, to measure binding of metals to a substrate and subsequent settling and T/DP-E Part 2, to assess the remobilization of metals following resuspension of substrate. The aim was to provide a standardized approach to assess binding to substrate through sorption and metal speciation changes and subsequent settling of the non-soluble particles, and the likelihood for remobilization of metals. This presentation outlines the method development and the rationale for each critical point in the methodology. Three substrates were selected to represent a low binding (LBS), high binding (HBS) and a substrate of intermediate composition based on the Fe and total organic carbon (TOC) concentrations as both are important factors in metal partitioning from the water column. 10 g/L of substrate was added to pH 6 aqueous medium containing 1 mg/L of the metal of interest. The substrate loading was based on calculations of the settling flux of solids to the sediment in a typical lake environment. The 1 mg/L metal loading was selected as it is equivalent to the highest loading for chronic classification. pH 6 was selected as it is typically a worst case for metal dissolution. 96 h experiments were conducted using the LBS and T/D solutions containing dissolved Ni, Co, Cu, Pb, Zn and 0.1 mg/L Ag. Results indicate that >70% removal is achieved for Cu, Pb and Ag within 96 h. 28 day tests with resuspension were conducted tracking dissolved and total Cu and Sr with additional measurements for Fe as a fingerprint of the substrate. 95% of Cu and 41% of Sr was removed within 28 days with no measurable increase on resuspension whereas both total and dissolved Fe increase at the resuspension event. An assessment of experimental repeatability indicates that the results obtained are reproducible.

**224 Modeling Metal Rapid Removal Experiments for Hazard Classification**

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In the context of hazard classification, the concept of degradability is an important consideration. For example, in the United Nations Globally Harmonized System (GHS) and European Union regulation on classification, labelling and packaging of substances and mixtures (CLP), less severe chronic classification entries are assigned to substances that are rapidly degraded (e.g. 70% degradation in 28 days). For metals, the concept of degradation as applied to organic chemicals has limited or no meaning. Recently, metal removal from the water column through partitioning/sedimentation and sequestration in sediment where proposed as a surrogate for degradation. A weight-of-evidence (WoE) approach was developed for assessment of metal removal from the water column. This approach relies upon an experimental component which is based upon existing standard methods OECD 29 (transformation/dissolution protocol) and OECD 308. The objective of the model development effort presented here is to provide mechanistic insight into important processes controlling removal from water column during the experiments performed using the OECD 29 and OECD 308 methods. A time-variable numerical model was developed in R using an explicit ordinary differential equation solver. The model was applied to datasets for copper, cobalt, strontium, silver, nickel, lead, and zinc. The OECD 29 and OECD 308 experimental data were successfully modeled with either a 2-layer (OECD 29) model or a 20-layer model (OECD 308). For the OECD 29 method, data for the various metals was described using a single (i.e., global) settling velocity, a single bulk exchange coefficient, and metal-specific kinetic adsorption rate constants and solid-solution distribution coefficients. The modeling analysis revealed that the important physical/chemical processes are kinetic adsorption of dissolved metals to particles, settling of particles and associated metals, and dissolved metal transport to and direct adsorption by the settled particles. For the OECD 308 protocol, metal transport via diffusion through bedded sediment layers played an important role in the loss of metal from the water column.

## Coupling Models with Monitoring Data and Future Perspectives for Exposure Assessment

**225 A Retrospective Review of Isocyanate Concentrations in Air and QC Samples from 3M Facilities Over the Last 6 years Using the ASSET EZ4-NCO Dry Sampler**

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As a global company, the 3M Environmental, Health, and Safety; EHS Laboratory is called upon to analyze both personnel and work area samples to determine the airborne isocyanate concentrations in many of our facilities throughout the world. Using the ASSET EZ4-NCO Dry Sampler, we have developed an approach to ensure sample and data quality integrity throughout the entire sample collection, storage, shipping and analytical process. This approach allows us to evaluate sample stability and integrity during the entire process, and assign analytical uncertainties to each sample value reported, reflective of the holistic process. This presentation will discuss the 3M EHS Laboratory approach to this entire sampling process, focusing on the Quality Control aspects, from initial request to delivery of the final report. A retrospective evaluation of the analytical recoveries from the ASSET EZ4-NCO Dry Sampler for 2,4-toluene diisocyanate (2,4-TDI), 2,6-toluene diisocyanate (2,6-TDI), and 4,4'-methylene diphenyl diisocyanate (4,4'-MDI) from all relevant Quality Control samples over a 6-year period will be presented. Over this 6-year period, the average analytical recoveries (n, standard deviation) of 2,4-TDI, 2,6-TDI and 4,4'-MDI are 108% (176, 12.9%), 104% (167, 13.1%) and 104% (212, 13.0%), respectively. In addition to the above results, the analytical recovery of the dibutylamine derivatives of these three isocyanates from ASSET EZ4-NCO Dry Samplers stored over a period of several months will be presented. The application of the analytical recovery from all Quality Control samples to assign an analytical uncertainty to all sample final results will be discussed, with examples given.

**226 Bayesian uncertainties analysis of modelled chemical exposure of global fish community: How can ChemTHEATRE improve the performance of FATE?**

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Numerical models such as Finely-Advanced Transboundary Environmental model (FATE) allow us to predict exposure of various environmental media (including atmosphere, oceans, soils, and biota) to chemicals, on a variety of spatiotemporal scales. However, there appear to be significant uncertainties in the model predictions, owing to those in the model input data and associated model parameterisations. Environmental monitoring datasets, against which the models are validated, have been largely limited in quantity and coverage, hence much effort is being devoted to data curation. Using a dataset curated on open-data platform ChemTHEATRE ('Chemicals' in the Tractable and Heuristic E-Archive for Traceability and Responsible-care Engagement), we evaluated the performance of FATE through a Bayesian uncertainties analysis in the light of uncertainties in the modelled exposure of global fish community to polychlorinated biphenyls (PCBs). Selected modelled processes to be discussed for this meeting are bioaccumulations in an Asia-Pacific ecosystem in the oceans, and exposure of sedimentary interfaces to PCBs, to improve model parameterisations and to better understand deep-sea mechanism that governs the fate and transport of PCBs, respectively.

**227 Evaluating the ability of probabilistic exposure models to predict sewage treatment plant effluent concentrations of chemicals disposed down the drain**

*J. Menzies, K. Casteel, The Procter & Gamble Co. / Environmental Stewardship and Sustainability Organization; K. Wehmeyer, P&G / Trace Analytical Core; K. McDonough, P&G / Environmental Stewardship*

Recent research has provided a better understanding of key parameters used in modelling the fate of consumer product ingredients disposed down the drain in the United States (US) including distributions describing per capita water use and sewer transit time. These distributions can be combined with laboratory based sewer and wastewater treatment plant (WWTP) simulation study results to create a high tier probabilistic model that predicts exposure concentrations in US WWTP effluents. The predicted concentrations can be compared with measured US WWTP effluent data in order to evaluate the strength of the model to predict actual environmental concentrations. Recent monitoring campaigns measured ingredient levels in the effluents of 40+ WWTPS across the US receiving >90% domestic wastewater. These domestic plants are expected to treat wastewater with the highest levels of consumer product ingredients. Case studies will include diethyldiester dimethyl ammonium chloride (DEEDMAC), amine oxide (AO) and linear alkyl benzene sulfonate (LAS). Recent effluent monitoring data will be compared with predicted effluent concentrations based on laboratory simulation studies in order to evaluate the ability of the model to predict actual environmental concentrations. In addition, this talk will discuss reasons for disparity between measured and monitored data and how to utilize all the information in a high tier exposure assessment.

**228 Pesticide surface water monitoring and ecohydrologic modeling to refine aquatic receptor exposure characterization.**

*P. Janney, J.J. Jenkins, Oregon State University / Environmental and Molecular Toxicology*

Pesticide surface water concentrations can be highly variable due to variation in climatic conditions, pesticide use associated agronomic practices, watershed geophysical and ecohydrologic characteristics. Pesticide surface water loading is a result of these stochastic processes leading to a range in concentrations that can vary on an hourly to seasonal time scale. Pesticide surface water monitoring is a deterministic approach to characterizing the stochasicity in pesticide surface water concentrations. Grab sampling provides point measures of exposure without an understanding of the temporal trends in exposure at toxicologically relevant time steps. Continuous sampling techniques, including passive sampling, can be useful in characterizing exposure on chronic time scales, however the variability of exposure concentrations on the acute time scale are normalized. Watershed scale ecohydrolgic models, including the Soil and Water Assessment Tool (SWAT), can be used to evaluate the connection between land management practices and pesticide surface water loading and assess pesticide surface water concentrations at appropriate time steps for exposure characterization. A modeling and monitoring approach was used to evaluate pesticide exposure in the Zollner Creek watershed located in the Willamette River Basin, Oregon. The Zollner Creek watershed contains designated critical habitat for two ESA listed Pacific salmonid species and has been intensively monitored since the early 1990s. SWAT model parameterization was based on realistic agronomic practices utilizing local expertise and probabilistic methods to characterize the spatial and temporal distribution of pesticide application practices. The probabilistic approach generated daily distributions of possible concentrations based on the characterization of the pesticide use practices. The daily distributions were evaluated to identify the most likely concentrations. The most likely concentration on a daily times step estimated by SWAT were compared with available grab sampling and passive sampling monitoring data to evaluate model performance. SWAT estimated daily data was also used to assess likely aquatic receptor pesticide exposure at acute and chronic time steps in relation to realistic pesticide application practices.

**229 Towards the development of a framework for applying non-target chemical analysis data within exposure and risk assessment**

*T. Gouin, TG Environmental Research / Safety and Environmental Assurance Centre; R. Parmar, ARC Arnot Research Consulting; J.A. Arnot, ARC Arnot Research & Consulting / Department of Physical & Environmental Science/Department of Pharmacology and Toxicology*

There is an increasing trend towards multi-targeted analysis and non-target screening methods to increase the number of analytes monitored in biomonitoring and environmental samples. While the opportunities from advances in chemical analytical capabilities have shown substantive development over recent years, application of information related to data reported from non-target analysis represents a challenge to the field of exposure modelling. For instance, there is no framework for interpreting and using data reported from studies involving non-target analysis to inform exposure and risk. The absence of guidance may consequently lead to difficulties in prioritizing substances for risk assessment or application in exposure estimation. In this study we examine the state of the science with respect to non-target analysis, and present a summary of the merits and limitations in risk prioritization and application in exposure assessment. These preliminary observations are then used to propose an initial framework for the appropriate use of non-target analysis data within exposure assessment. The recommendations concludes with a number of suggestions regarding how these data can be better gathered and reported in order to strengthen their applications for chemical risk assessment, including emerging contaminants.

**230 Elucidating the dominant pathways of human exposure to chemicals used indoors**

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The general population is exposed to a variety of chemicals used indoors such as plasticizers and flame retardants. Once released into the indoor environment, these chemicals can be taken up by humans via inhalation, dermal permeation or hand-to-mouth contact (non-dietary ingestion) resulting in "near-field" human exposure. Some of these chemicals are also able to migrate to urban and rural areas and enter human food chains; consumption of contaminated food then leads to "far-field" human exposure. In the context of multi-pathway exposure, identifying the dominant exposure pathway(s) is essential for minimizing human exposure and potential adverse effects. In this work, we investigate the relative importance of near- and far-field exposure routes of chemicals used indoors, using a human exposure model that incorporates multimedia chemical fate in the indoor-urban-rural continuum and bioaccumulation along terrestrial and aquatic food chains. The model is applied to simulate exposure of Swedish and Canadian populations to sets of hypothetical chemicals with different partitioning properties, persistence in environmental matrices, and biotransformation rates in organisms. All chemicals are assumed to be used and released entirely indoors. We find that, despite being released indoors, exposure to persistent chemicals with a log $K_{OW}$ between 6 and 11 and a log $K_{OA} > 6$ (e.g., higher substituted polychlorinated biphenyls and polybrominated diphenyl ethers) tends to occur primarily by dietary ingestion, whereas exposure to chemicals with a log $K_{OA} < 6$ (e.g., cyclic volatile methylsiloxanes) is likely to be dominated by inhalation of indoor air. If a compound can be readily degraded in the environment and within organisms, the contribution of dietary uptake decreases whereas that of indoor inhalation and non-dietary ingestion increases. Compared with Swedes, Canadians consume more terrestrial food (e.g., beef and dairy), thus the dietary contribution to their total exposure to chemicals with a log $K_{OA} > 6$ and a log $K_{OW}$ between 2 and 6 (e.g., phthalates) is relatively higher. This work, for the first time, provides a systematic overview of pathway-specific contributions to aggregate human exposure to the universe of chemicals used indoors.

**231 Responses of Mercury Deposition and Bioaccumulation in the Great Lakes Region to Policy and Other Large-scale Drivers of Emissions**

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Anthropogenic emissions combined with long-range atmospheric transport and deposition have contributed to a state-wide fish consumption advisory for mercury (Hg) in Michigan as in 35 other states in the U.S. Of ~70 lakes in Michigan's Upper Peninsula (UP) where walleye Hg has been measured, nearly 80% had concentrations above 0.3 ppm, USEPA's criterion value. Mercury (Hg) emissions pose a global problem; however, neither emissions nor regulations are uniform world-wide, and hence the impacts of regulations are also likely to vary regionally. We report here an approach to model the effectiveness of regulations at different scales (local, regional, global) in reducing Hg deposition and fish Hg concentrations in the Laurentian Great Lakes (GL) region. The potential effects of global change on deposition are also modeled. We focus on one of the most vulnerable communities within the region, a tribe in Michigan's Upper Peninsula (UP) with a high fish consumption rate. For the GL region, elements of global change (climate, biomass burning, land use) are projected to have modest impacts (< 5% change from the year 2000 to 2050) on Hg deposition. For this region, our estimate of the effects of elimination of anthropogenic emissions is a 70% decrease in deposition, while our minimal regulation scenario increases emissions by 35%. Existing policies have the potential to reduce deposition by 20%, with most of the reduction attributable to U.S. policies. Local policies within the GL region show little effect, and global policy as embedded in the Minamata Convention is projected to decrease deposition by approximately 3%. Even within the GL region, effects of policy are not uniform; areas close to emission sources (Illinois, Indiana, Ohio, Pennsylvania) experience larger decreases in deposition than other areas including Michigan's UP. The UP landscape is highly sensitive to Hg deposition. Sensitivity to mercury is caused primarily by the region's abundant wetlands. None of the modeled policy scenarios are projected to reduce fish Hg concentrations to the target that would be safe for the local tribe. Regions like Michigan's UP that are highly sensitive to mercury deposition and that will see little reduction in deposition due to regulations require more aggressive policies to reduce emissions to achieve recovery. We highlight scientific uncertainties that continue to limit our ability to accurately predict fish Hg changes over time.

**232 Who in the world is most exposed to PCBs? Using models to identify highly exposed populations**

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Human subpopulations experience different exposure to persistent organic pollutants (POPs) because of differences in the structure of their food webs and the extent of environmental contamination. We quantified the time-variant exposure of different human populations around the world to one representative POP, namely the polychlorinated biphenyl (PCB) congener 153, based on a dynamic simulation of both global environmental fate (using the model BETR-Global) and human food chain bioaccumulation (using the model ACC-HUMAN). The approach identified subpopulations whose diets include a warm-blooded carnivore (such as the Inuit eating marine mammals) as experiencing the world's highest PCB-153 exposure, i.e. the very large biomagnification potential of their food web more than makes up for the remoteness of their living environment. The results that Inuit eating a traditional diet are most exposed to PCB-153 is in agreement with empirical observations, as concentrations comparable or greater than those measured in Inuit have only been measured in people living close to major PCB point sources, who have been subject to occupational exposure, or who have been poisoned. For subpopulations that do not eat warm-blooded carnivores, the proximity to sources of PCBs is more important than food web structure and environmental conditions for differentiating their exposure to PCBs. Indeed, a worldwide survey of concentrations in human breast milk identifies exclusively central and Eastern European countries as having elevated PCB concentrations, consistent with the intensity of PCB emissions in this region. Concentrations in breast milk in countries in tropical latitudes and the Southern hemispheric are generally much lower, again reflective of the global distribution of historical PCB emissions.

## Non-Targeted Analysis: Comparing ENTACT Results and Assessing Informatics Approaches

**233 Study Design and Initial Results for EPA's Non-Targeted Analysis Collaborative Trial (ENTACT)**

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Non-targeted analysis (NTA) is a relatively new technique that strives to identify as many compounds present in a sample as possible, providing mostly qualitative results. NTA is useful to fill knowledge gaps about the many environmental chemicals posing exposure potential. However, most NTA approaches are in their infancy, with variable quality control and poor understanding of the domain of applicability. EPA's Non-Targeted Analysis Collaborative Trial (ENTACT) is designed to address these challenges through a controlled study involving multiple laboratories using different approaches on identical known, but blinded, samples. This presentation will cover the study design for ENTACT, specific results from the EPA analyses, and preliminary results from other participating laboratories (30 total). ENTACT samples include 10 synthetic mixtures containing 95-365 chemicals each, 3 exposure-related media extracts (unaltered and fortified with a mixture), and single chemical standards. Participating laboratories were initially blinded to the contents of the samples. After submission of initial results, participants are unblinded to fully evaluate their performance and allow adjustments to their workflow to improve results. About half the participants have submitted results using a variety of chromatography and mass spectrometry (MS) platforms. EPA analyses with liquid chromatography (LC) time-of-flight MS detected 60% of spiked substances in the mixtures, with true positive rates reaching a maximum of 68% after unblinding. Reproducibility rates averaged 75% for compounds repeated in multiple mixtures. Across a broader range of participants, method comparison between gas chromatography (GC) and LC showed that of 1,269 unique substances in the mixtures, 195 were not detected by either method; 809 were detected by GC (378 only GC), and 801 were detected by LC (265 only LC). Statistical separation of the GC-only, LC-only, and non-detected substance sets (Kruskal-Wallace non-parametric test, $P < 0.05$) based on 8 physico-chemical properties (BP, MP, $K_{oa}$, $K_{oc}$, $K_{ow}$, Henry's Law, VP, and water solubility) predicted from the OPEn quantitative structure-activity Relationship Application (OPERA) suggests differences in chemical space coverage among the 3 sets. ENTACT is the first study of its kind to objectively evaluate the performance of NTA methods on a common set of chemicals, providing a benchmark for current methods, and revealing areas for improvement and development.

**234 Comprehensive, Non-Target Characterization of Environmental Exposome Samples Using GCxGC & High Resolution Time of Flight Mass Spectrometry**

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Historically targeted analysis has been the primary route to evaluate complex environmental samples. This constrained testing, while effective has often missed emerging or unexpected compounds within samples. Recent improvements in detection and data processing capabilities of various systems have allowed scientists to more fully evaluate these same samples using non-targeted (NT) techniques. As a result, the EPA is currently conducting a multiple lab, multiple platform evaluation for non-targeted analysis methods in samples designed to mimic the environmental exposome. The project contains two initial phases, first a blinded study is conducted and reported. In phase two the individual standard component lists are provided and the evaluation revised as necessary. Each blind standard is reported to contain between 100-400 spiked analytes with potential for more due to contaminants, intra-sample degradation or reaction product formation. This presentation describes the systematic logic used for identification of the unknowns, its results and the lessons learned from the process as it applied to the first round of ten, blinded ENTACT samples for a single platform. The platform used was a comprehensive GCxGC gas chromatograph coupled with a high resolution accurate mass (HRAM) time of flight mass spectrometer (TOF-MS) in both electron ionization (EI) & chemical ionization (CI) modes. Deconvolved spectra were matched to existing commercial MS libraries and screened based on the peak's retention index value, molecular ion mass accuracy and fragment ion formula fidelity. Questions addressed will be : (i) what percentage of each sample was correctly identified, (ii) what instrumentation characteristics contributed most significantly to the identification and (iii) what impurities, reaction products and degradation products were identified.

**235 Comparison of different ionization sources in the detection of unknown compounds in environmental samples within the EPA ENTACT project**

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The development of non-target screening methods to assess environmental contaminants of emerging concern, which are not commonly monitored for, is of utmost importance especially when there is no previous knowledge on the identity of the source of pollution. The advances in the field of high-resolution mass spectrometry offers great sensitivity and resolution, which coupled to ultra-high performance liquid chromatography represents a powerful tool for the separation and detection of a very large number of organic contaminants. Nowadays, the most commonly used ionization sources in LC-MS/MS are electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). Although ESI is the most applied technique for polar and higher molecular weight compounds, APCI has been shown some advantages over ESI, especially in the ionization of thermally stable polar and non-polar compounds. The ionization process associated with APCI is considered more efficient and energetic than ESI, where gas phase reactions may generate more fragment ions relative to the parent ion. Also, APCI is less susceptible to signal suppression and matrix interferences than ESI. For certain classes of compounds that are traditionally very difficult to ionize or tend to show low sensitivity in LC-MS/MS, APCI has provided increased sensitivity for these 'tough to ionize' compounds, being considered a complementary technique to ESI. APCI has been reported to be more sensitive than ESI for triazines, phenylurea herbicides, biphenols, polyaromatic hydrocarbons (PAHs) and organochlorine pesticides. In this study, we have compared ESI and APCI for the detection of unknown compounds in 10 spiked samples (mixtures) as part of the inter-laboratorial study ENTACT conducted by the Environmental Protection Agency (EPA). Preliminary blinded results, without extensive manual processing, have shown the detection of 7911 features in APCI and 6428 features on HESI, with 2399 features in common to both ionization techniques. Kendrick mass plots and van Krevelen diagrams suggest that APCI was able to detect more chlorinated compounds and compounds with higher H:C and N:C ratio than ESI. Unblinded results will better elucidate the real capability of the methods in non-targeted analysis. In addition, the different ionizations sources will be also compared in real environmental samples (water samples collected from South Florida) regarding the detection and identification of unknown compounds.

**236 An Overview of Performance of the USEPA's Non-Targeted Analysis Collaborative Trial (ENTACT) Using LC-QTOF-HRMS**

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USEPA's ENTACT project was designed to facilitate international collaboration and comparison of analysis protocols across laboratories in the non-targeted analysis, emerging paradigm of environmental pollutants monitoring. In early 2017 we received ENTACT samples composed of 10 standard mixtures and 6 fortified sample extracts of wrist band, human serum and house dust. Upon completion of instrumental and data analysis, we submitted final report in September 2017. Thereafter, we received the key file and compared our submitted results and provided the evaluation summary. The samples were analyzed in both positive and negative electrospray ionization (ESI) modes using Agilent 6500 LC-QTOF-HRMS. Molecular features were extracted from raw data using commercially available software (Agilent Masshunter Qual). Features were aligned and quality control parameters were set to filter the features by peak intensity and subtraction of laboratory blank backgrounds using Agilent Mass Profiler Professional. The validated features were screened against the MS Ready library provided by USEPA. Matching results were further evaluated and validated, with a combined evaluation of matching score, retention times and structures. For the evaluation, we assigned three levels of positive identification to the reported compounds: one star for only accurate mass, two stars for mass and formula, three stars for mass, formula and structure. Overall, 53-70% of the spiked compounds were reported at one and two stars level of identification, and 33-63% spiked compounds were reported for three stars level. We identified a wide range of compounds with very different physical and chemical properties. We also curated those compounds that were classified as non-reported, and LC amenability was identified as the major reason. We also build libraries (negative and positive) by combining our experimental data with input from the key files, and additional information from the EPA CompTox Chemistry Dashboard. In this presentation, the instrument analysis method, data work flow, the results evaluation, and limitations will be discussed. The views expressed herein are those of the authors and do not necessarily reflect those of the California Department of Toxic Substances Control.

**237 Probability Assessment for Identification in Non-Targeted Environmental Research (PAINTER): A new workflow for pollutant annotation and prioritization**

*L. Ferguson, Duke University / Nicholas School of the Environment Department of Civil and Environmental Engineering; G.J. Getzinger, ETH Zurich / Department of Environmental Systems Science*

High-resolution accurate-mass mass spectrometry (HR/AM MS) is capable of comprehensive structural elucidation of numerous trace-level organic contaminants in the environment. However, comprehensive sample characterization is made difficult by inefficiencies in the annotation and prioritization of detected features and postulated structures based on tandem MS analysis. We have developed a holistic, non-targeted analysis workflow to address these challenges, which combines ultra-high resolution tandem mass spectrometry, cheminformatics, and computational mass spectrometry. We have assessed and validated this approach through analysis of formulated chemical mixtures and serum, dust, and silicone wristband extracts as part of the EPA ENTACT collaborative trial. Samples were analyzed using an Orbitrap Fusion Lumos tribrid tandem LC-HR/AM mass spectrometer in ESI and APCI/APPI modes, in both positive and negative polarity. After feature detection, componentization, and alignment, custom data processing scripts were deployed to pipe relevant feature data to computational mass spectrometry tools for molecular formula assignment based on isotope pattern and fragment spectrum decomposition (SIRIUS, formulas assigned to >90% of features), harmonized spectral library searching routines (library size: 23,000 unique compounds) and structure assignment from the PubChem (database size: 65 million compounds) compound repository (postulated structures returned for >80% of features) and automated tandem mass spectral annotation and scoring (MetFrag CL, MAGMa, CSI:FingerID, and CFM-ID). A novel machine learning-based approach was developed and deployed to provide probability-based compound identification assessment from in silico MS/MS predictions, compound metadata (patents, references, and sources), and presence in prioritized compound database lists. After rigorous data filtering, organic compounds in samples and mixtures were tentatively identified according to an expanded Schymanski confidence scale, representing classes of pharmaceuticals, transformation products, consumer chemicals, and industrial compounds. Overall, our results highlight the benefit of combining of state-of-the-art computational mass spectrometry tools with chemoinformatic approaches for increased efficiency and annotation rate in non-targeted analysis approaches.

**238 Improvement of Mass Accuracy to Sub-ppm via Putative Lock Mass Algorithm (PLMA)**

*S. Kutarna, University of Toronto; H. Peng, University of Saskatchewan / Department of Chemistry*

Non-targeted analysis (NTA) using high resolution mass spectrometry (HRMS) provides a promising strategy to screen unknown compounds in complicated environmental matrices. For HRMS-based NTA methods, mass accuracy is the one of the most critical parameters used for the identification of environmental compounds. Preliminary studies found that the mass shift of HRMS showed large variations (>5 ppm) across different runs, or even between different retention times in the same run. This variation in mass shift increases the false discovery rate for NTA. The present study was motivated by the discovery of ubiquitous background contaminants in samples analyzed by HPLC-HRMS, which were identified as fatty acids or humic acids, present in the mobile phase as impurities. An algorithm was proposed to search all ions in the MS1 spectra (100-800 m/z) by 0.001 bins across all retention times. The background contaminants which could be detected across all retention times were selected as potential lock masses. Then, curve fitting was iteratively conducted to identify the optimal function to simulate the mass shift across different mass ranges. The m/z values for all ions in the spectrum were calibrated by the established calibration curves. Using this Putative Lock Mass Algorithm (PLMA), the mass accuracy of an Orbitrap mass spectrometer was improved from >5 ppm to sub-ppm accuracy. The PLMA was then successfully applied to NTA with different instruments (Q-Exactive, Q-Exactive HF) and environmental matrices (i.e. drinking water, house dust, and sediments). This algorithm will have important and broad applications for NTA in future studies.

**239 An Integrated Approach for Bio-effect Prediction based on Non-targeted Chemical Analysis**

*N. Kumar, J. Crago, Texas Tech University / Environmental Toxicology The Institute of Environmental and Human Health (TIEHH)*

Contaminants of emerging concern (CEC) such as pharmaceuticals and personal care products (PPCPs), flame retardants, industrial chemicals, pesticides, and their transformed products are entering the environment. Therefore, the environmental risk assessment of complex chemical mixtures requires an integration of analytical toxicology, molecular toxicology, and bioinformatics. The goal of this study is to integrate information from non-targeted chemical analysis of water samples, predict target(s) for chemicals with bioinformatic tools such as curated (Comparative Toxicogenomic Database, CTD) and non-curated databases (Similarity Ensemble Approach, SEA), and confirm predicted adverse outcomes with molecular techniques. Water samples were collected from 4 locations of Lubbock Canyon Lake System, which is dominated by wastewater treatment plant (WWTP) effluent. For example, a non-targeted chemical analysis (UPLC-orbitrap) was performed, and 4,423 unique structures were identified with the METLIN database in a water sample which was collected at approx. 7 miles downstream of the WWTP discharge point. 84.81% of identified structures were found in the USEPA CompTox database. Among unique identified compounds; 244 compounds were present in ExpoCast and 215 compounds were present in Tox21 Screening Library. The fathead minnow acute toxicity data was available for 31 compounds. Chemical gene interactions were identified with the SEA and CTD databases and a pathway analysis was performed using gene ontology (http://geneontology.org/). The top three pathways were Gonadotropin-releasing hormone receptor pathway, angiogenesis, and neuronal signaling disruption. The zebrafish *(Danio rerio*) model will be used to confirm the predicted toxicity. This presentation will outline the spatial and temporal distribution of CECs and their adverse effect on aquatic organisms in the Lubbock Canyon Lake System. Additionally, a workflow from non-targeted chemical analysis to bio-effect prediction will be presented.

## Existing, Emerging and New Chemical Contaminants in Changing Polar Environments

**240 What can Arctic seabirds tell us about changing emission patterns of contaminants?**

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Environmental chemical contaminants are a global problem, and their presence in the Arctic reflects the integrated distribution processes and the way the Arctic interacts with the rest of the world. By examining contaminant levels in wildlife such as seabirds, we can look for new contaminants in the Arctic. Sentinel wildlife species, such as seabirds, can also be used to determine whether existing chemical contaminants of concern are increasing or decreasing, and whether those trends are being impacted by changing weather and climate conditions. Eggs of thick-billed murres (*Uria lomvia*), northern fulmars (*Fulmarus glacialis*) and black-legged kittiwakes (*Rissa tridactyla*) breeding on Prince Leopold Island in Lancaster Sound in the Canadian Arctic have been monitored for environmental contaminants since 1975. Concentrations of most legacy organochlorines such as the polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) have declined dramatically in arctic seabird eggs over the past 40 years in response to national and international regulations or bans. Likewise, concentrations of polychorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) have also declined, as have polychlorinated napthalenes (PCNs). Brominated flame retardants, such as polybrominated diphenyl ethers (PBDEs), increased in eggs of thick-billed murres and northern fulmars between 1975 and 2003 after which time levels declined, likely reflecting the phase-out of certain technical PBDE-containing products. Concentrations of poly- and per-fluorinated alkyl substances (PFASs) in murre and fulmar eggs remained unchanged (e.g. perfluorooctane sulfonate – PFOS) or increased (e.g. perfluorinated carboxylates- PFCAs) for several decades but levels may now have started to decline. We examine the temporal trends of these contaminant groups in arctic seabird eggs in the context of emissions and initiatives to reduce those emissions, as well as the potential impact that climate change may have on those trends.

**241 Sources, bioaccumulation, and biomagnification of PFASs in the ringed seal foodweb of Lake Melville, Northern Labrador, Canada**

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Perfluoroalkyl substances (PFASs) are a class of synthetic organic compounds that are highly persistent in the environment and can cause adverse health effects (e.g., endocrine disruption, immunosuppression, and cancer) in humans and wildlife. PFASs can be transported to polar regions via atmospheric oxidation of volatile precursors and ocean circulation. Due to their hydrophobic, lipophobic, and hydrophilic properties, PFASs may bioaccumulate in protein-rich tissues of organisms and biomagnify in foodwebs of remote ecosystems. One such remote ecosystem is Lake Melville, an estuarine fjord in northern Labrador, Canada, that receives both freshwater inputs (e.g., from the lower Churchill River) and marine water from the Labrador Sea. This region is currently undergoing environmental changes including climate warming and reservoir creation for hydroelectric power development at Muskrat Falls on the lower Churchill River. Consequently, PFASs levels to the local ringed seal (Phoca hispida) foodweb may increase, posing potential health concerns for local indigenous people who rely on ringed seals, fish, and other traditional country foods for sustenance. The objectives of this research are to identify environmental sources of PFASs and assess their bioaccumulation and biomagnification throughout the Lake Melville foodweb. Seal samples were collected between 2013-2017 by hunters during local harvests. Lower foodweb and water samples were collected between 2014-2017. Samples of seal and fish muscle and liver, water, plankton, and invertebrates were chemically extracted then analyzed for PFAS concentrations using UPLC-MS/MS. Principal Component Analysis of PFASs concentrations in water samples was used to identify possible sources of PFASs to this region, with results suggesting that short-chain and long-chain PFASs in Lake Melville may come from different sources. Calculation of bioaccumulation factors (BAF) in zooplankton, invertebrates, fish, and ringed seals demonstrates that certain PFASs bioaccumulate in the local foodweb. Stable isotope ratios ($\delta$13C and $\delta$15N) of foodweb samples and trophic biomagnification factor (TMF) will be determined to assess biomagnification of PFASs in the ringed seal foodweb. This study is the first to investigate PFASs in the ringed seal foodweb in Labrador and will shed light upon whether the levels of PFASs in ringed seals and other biota are of potential concern for the health of Northern Indigenous people.

**242 Contaminant concentrations are related to climate and weather factors in the Hudson Bay: A cross-species comparison**

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Wildlife from the Hudson Bay Region of Canada typically have among the greatest concentrations of persistent organic pollutants (POPs) and mercury (total mercury = THg) in the Canadian Arctic, and may be experiencing exacerbated climate change at their relatively low latitude ranges. In the present study, relationships between contaminants and weather/climate variables were investigated in polar bears (*Ursus maritimus*) from southern and western Hudson Bay (SHB and WHB), thick-billed murre (TBMU; *Uria lomvia*) from Coats Island, and caribou (*Rangifer tarandus*) from a WHB regional herd. THg was assessed in all species while POPs, including a–hexachlorocyclohexane (a–HCH), *p,p′*-dichlorodiphenyltrichloroethane (*p,p′*-DDE), 2,2′,4,4′,5,5′-hexachlorobiphenyl (CB-153), 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) and perfluorooctane sulfonate (PFOS), were assessed in bears and TBMU. Data were compiled from the early 1990’s (WHB bears, TBMU) or mid-2000’s (caribou, SHB bears) up to 2015. Annual and seasonal climate/weather factors included air temperatures, wind speeds, air pressure, precipitation, ice conditions (break-up, freeze-up, ice-free days), and the Arctic and North Atlantic Oscillation Indices (AO and NAO respectively). General linear models (GLMs) showed that caribou THg concentrations increased with increasing NAO (annual and spring) and AO (spring), and with earlier sea-ice breakup. THg models for WHB bears and TBMU showed that concentrations decreased with increasing spring and/or winter wind speeds. a-HCH concentrations decreased with an increasing, 1-year time lagged AO (June) in TBMU, while the concentrations decreased with increasing ice-free days in polar bears. In TBMU and polar bears, *p,p’*-DDE increased with increasing air pressures or decreased with increasing precipitation. Concentrations of BDE-47 increased with increasing precipitation (annual) in TBMU, while decreasing with increasing winter precipitation in WHB bears. A 1-year lagged AO (fall) produced the best model for CB-153 in polar bears, while the lagged NAO (fall and summer) was most significant for TBMU (sea-ice conditions were also significant for both). PFOS decreased with increasing annual and spring land surface temperatures. Some similarities were evident; however there were both distinct and subtle differences in the relationships observed that suggest that climate change may differentially affect contaminant trends in these species.

**243 Evidence for microplastic contamination in remote Canadian Arctic archipelago marine sediments**

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Plastics are an integral part of contemporary society. Researchers have estimated that 8300 million tonnes of plastic were manufactured by 2016, of which 6300 million tonnes have ended up as waste. Single-use sources, such as bags and packaging, are an important contribution to this waste. Plastic waste degrades or fragments to become microplastics, which are plastic particles of < 5 mm. Microplastics are a concern due to the threat posed to terrestrial and aquatic organisms when ingested, which may then be transferred through the food web to higher trophic levels. Evidence continues to emerge showing the ubiquitous distributions of microplastics in the environment, and there is limited but growing evidence showing their presence in remote Arctic environments. The presence and impact of microplastics in the Arctic may be exacerbated by climate change, through melting of multi-year sea ice releasing previously contained microplastics, and increased ship transportation in the Arctic. Our research aims to determine the extent of microplastic contamination across the Canadian Arctic archipelago marine system, and to evaluate sources and transport pathways of the microplastics. Fourteen surface sediment samples were collected between 2014 and 2017 across approximately 3,300 km of the Canadian Arctic archipelago marine system and from water column depths ranging from 40 to ~1500 m, as a part of ArcticNet. The surface sediments were examined for microplastic particles > 20 µm, specifically particle abundance, distribution and characteristics. Our data indicate the presence of microplastic particles in all 14 samples. However, abundance of particles varies across sites. In the size fraction > 500 µm, microplastic abundance varies by an order of magnitude (from 360 to 5,000 particles/kg dried sediment). The greatest abundance of microparticles > 500 µm was found in the western-most sample located in the Beaufort Sea just offshore of Alaska. The lowest abundance was found in a sample located in the eastern Canadian Arctic, in the Davis Strait just offshore Baffin Island. Particle types were mostly fibers, with fragments as the second most common particle type. Our results indicate the ubiquity of microplastics across the Canadian Arctic Ocean, and that microplastics likely undergo long-range transport in marine systems, but that there is also the potential for local sources even in remote environments.

**244 OCPs and PCBs in the terrestrial environment of Schirmacher Hills, Antarctica: Environmental assessment and degradation**

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Antarctica has been historically considered a remote and pristine continent. However, long-range transport of pollutants from other continents, and local activities in research stations and the Antarctic tourism industry have introduced many contaminants in the region. In this work, we report the occurrence of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) in mosses, soil and water from the Schirmacher Hills, Dronning Maud Land, Antarctica (latitude 70°43′50"S–70°46′40"S, longitude 11°22′40"E–11°54′25"E). This is the first study in the region after the ban on production and consumption of the legacy POPs. We present results on the occurrence of PCBs and OCPs in the terrestrial environment of Schirmacher Hills, Antarctica, and direct PCB release in the region, and the degradation kinetics of PCB congeners. Out of nine evaluated OCPs [α-hexachlorohexane (HCH), β-HCH, γ-HCH, δ-HCH, α-Endosulfan, β-Endosulfan, Endosulfan sulfate, *p,p′*-dichlorodiphenyldichloroethylene (DDE) and *p,p′*-dichlorodiphenyldichloroethane (DDD)], only α-HCH and *p,p′*-DDE were detected in moss. α-HCH concentrations (4.48 ng/g dw) were comparable to other locations while *p,p′*-DDE (31.2 ng/g dw) concentrations are higher than those observed by 5 times or more. None of the OCPs were detected in soil. Out of the tested 28 polychlorinated biphenyl (PCB) congeners, only 6 PCBs were detected. $\Sigma_{28}$PCBs in both moss (122 ± 115 ng/g dw, $n = 5$] and water (30 ng/L and 165 ng/L, $n = 2$) are higher by up to 10 times compared to other studies around the continent. Heavier congeners (hexa through nona) in both moss and water samples constitute 52–100% of $\Sigma_{28}$PCBs. This suggests that some localized sources of PCBs may still exist in the Schirmacher Hills region. It is possible that the old research stations in the area may be one of those sources. We find that while the observed congener distribution can be expained using congener distributions in known commercial PCB mixtures (Aroclors, Sovol and Clophen) in some samples, a post-depostition transformation in snow/ice (in glaciers) is required to explain the remaining observations.

**245 Interactions of persistent organic pollutants with vitamins A and E in the blubber of killer whales moving into Arctic waters**

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Along with Arctic sea ice contracting both spatially and temporally, North Atlantic killer whales (*Orcinus orca*) appear to have expanded their habitat ranges into Arctic regions. In the Canadian Arctic and Greenland, killer whales have been observed hunting marine mammals, which may represent a shift from their presumably fish-based diet in North Atlantic waters. Consistent with this, we recently showed that 18 individuals sampled in Greenland from 2012-2014 appeared to have high blubber levels of biomagnifying persistent organic pollutants (POPs) [e.g. ΣPCB: 71.6 ± 18.4 mg $kg^{-1}$ lipid weight (lw)] relative to conspecifics stranded in the North Atlantic in 2008 (ΣPCB: 5.2 ± 1.7 mg $kg^{-1}$ lw). About 67% of the Greenland killer whales showed PCB levels exceeding threshold of effects of 41 mg $kg^{-1}$ lw reported for other marine mammals. To further examine the potential consequences of these high POP levels in the Greenland killer whales, we evaluated the levels of vitamins A and E in the blubber of a subset ($n = 15$) of these individuals and examined the relationship with POP levels. Due to lipid stratification that occurs within marine mammal blubber, we additionally compared levels of these lipophilic vitamins and POPs between the innermost and outermost layers of the blubber. Blubber levels of vitamin A (measured as retinol) averaged 32.8 ± 4.6 µg $g^{-1}$ wet weight (ww) ~72.9 ± 12.1 µg $g^{-1}$ lw, and levels of vitamin E (measured as α-tocopherol) averaged 34.1 ± 4.4 µg $g^{-1}$ ww ~75.0 ± 12.0 µg $g^{-1}$ lw. Preliminary results suggested similar levels of vitamin A between inner and outer blubber layers, while levels of vitamin E were significantly higher in inner compared to outer layers (both at lw and ww basis, $p < 0.001$). Moreover, levels of vitamin A were similar among sex/age classes, while ww levels of vitamin E were higher in adult females compared to subadults ($p = 0.01$), but similar at lw basis. After accounting for this variation related to blubber layer and sex/age class, as well as diet based on fatty acid signatures, we will perform an assessment of the relationship of blubber vitamin A and E levels to blubber POP levels. We will eventually use the results to estimate a toxicity reference value for vitamin A and E disruption in killer whales. Such value allows further refinement in the assessment of risks posed by POPs for these high trophic level marine predators, which may be critical as their exposures change due to entry into novel Arctic habitats.

**246 "New" PCBs in the Arctic: New surface snow measurements from Svalbard**

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We contributed "New unintentionally generated PCBs in the Arctic" for the Arctic Monitoring Assessment Program's (AMAP) 2016 Assessment: Chemicals of Emerging Arctic Concern (CEAC), presenting some unresolved anomalies and the difficulty assessing the proportion of PCBs observed from long-range transport and local sources on Svalbard. Since then we have analyzed 209 PCB congeners in surface snows in four sites across Svalbard using EPA method 1668. Unintentionally produced PCB-11 is consistently measured in high proportions globally and more recently in polar observations. For AMAP we reported the Garmash et al. (2013) time line of ice core measurements from the Lomonosovfonna glacier on Svalbard from 1957-2009 with estimated PCB-11 fluxes of 0.18-0.45 pg $cm^{-2}$ $yr^{1}$. Surface snow samples showed a PCB-11 flux of 0.83 pg $cm^{-2}$ $yr^{1}$, 2009–2010. Variations in measurements over time and ice cores vs surface snow may arise from surface air exchange (volatilization) of the lower molecular mass and more volatile PCB congeners (mono's, di's and tri's) during summer. While most of the research has focused on the origin of PCB-11 and other unintentionally produced PCB from pigment, thermal sources are also a possible source. Surface snow samples were collected from four glaciers on Svalbard representing the winter of 2013-2014 at Austfonna, Holtedahlfonna, Kongsvegen and Lomonosovfonna. The sites are maximum 220 km apart west to east (Holtedahlfonna to Austfonna). PCB-11 is prominent in all of the samples. Kongsvegen is distinguished with the lowest flux of PCB-11 (0.50 pg $cm^{-2}$ $yr^{1}$), while the other three sites have nearly equal inputs of about 0.72 pg $cm^{-2}$ $yr^{1}$. EPA method 1668 enables us to distinguish PCB-5 from other congeners (PCB-8 for example), providing new questions about sources. We see PCB-5 in all samples, ranging from 1% – 3% of total PCB with an declining flux trend from west to east: Kongsvegen (0.71 pg $cm^{-2}$ $yr^{1}$), Holtedahlfonna, (0.60 pg $cm^{-2}$ $yr^{1}$), Lomonosovfonna, 0.32 pg $cm^{-2}$ $yr^{1}$, and at Austfonna, 0.11 pg $cm^{-2}$ $yr^{1}$, suggesting that PCB-5, and some other PCB congeners at western Svalbard sites may be from local sources. PCB-5 was not produced in Aroclor mixtures (USA, UK), or in Clophen (Germany). We are investigating the possibility that PCB-5 was found in the Russian product "Trichlorodiphenyl" (TCD).

**247 Organophosphate Esters Flame Retardants and Plasticizers in Arctic Waters**

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Organophosphate ester (OPEs) flame retardants and plasticizers are receiving increased attention due to their high levels in indoor air and dust and presence in remote locations including the Canadian Arctic. The widespread global distribution of OPEs is believed to arrive through various mechanisms including oceanic transport and air transport in the gas phase and on fine particles that undergo disposition in the colder arctic environment. Water samples were collected throughout the Canadian Arctic between 2013-2017 as part of ArcticNet and the Northern Contaminants Program. Samples were mainly taken from on board the CCGS Amundsen but also at a land based station in Resolute Bay, NT and Daneborg, Greenland. Levels were very high compared to other flame retardants including PBDEs. OPEs most frequently detected in the arctic environment were tri-phenyl phosphate (TPhP), tris(2-chloroethyl) phosphate (TCEP), tris(2-chloroisopropyl) phosphate (TCPP) and tris(1,3-dichloro-2-propyl) phosphate (TDCPP). Some OPE compounds are becoming more frequently detected in arctic waters, this includes ethyl-hexyl diphenyl phosphate (EHDPP), tri-cresyl phosphate (TCP) and tris(2-isopropylphenyl) phosphate (T2iPPP). We found levels were lower on the eastern side of the Canadian archipelago and higher in the Beaufort Sea. Iso-propylated and tert-butyl diphenyl organophosphates were also found in the arctic environment. We will present spatial trends within the Canadian arctic as samples spanned a west to east transect from the Beaufort Sea to Baffin Bay and identify possible sources.

## Advancing Fate and Effects Modeling and Their Integration to Increase the Relevance and Robustness of Risk Assessments

**248 Identifying important drivers in exposure and effects mitigation assessments: A general global sensitivity and uncertainty analysis framework**

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Long-term environmental risk assessments (RA) involve the combination of complicated deterministic models to ascertain regulatory compliance. The modeling framework often involves large sets of input factors (model parameters, initial and boundary conditions, and other model structure options). How can we identify the relative importance of human, chemical, physical and biological drivers on the assessment results? Is there a case for "the right answers for the right reasons"? For the case of mitigation practices like vegetative filter strips (VFS) for runoff mitigation, what are the important factors controlling or limiting their field efficiency under different field settings? Global sensitivity and uncertainty analysis (GSUA) represents a powerful and flexible framework to evaluate the uncertainty of the assessments, the relative importance of the factors controlling the results, and their predominant contribution to the model output (direct effects, interactions, non-linearities, and directionality). We present the GSUA framework and its application to the case of runoff mitigation with VFS to test alternative hypothesis on the relative effects that different field conditions can have on the assessments with and without this field practice. We evaluate the combination of the current EPA RA in combination with VFSMOD, an established numerical model for the analysis of runoff, sediment, and pesticide transport in VFS. We present a systematic study of the importance of different field conditions that have been proposed in the past as limiting the efficiency of VFS in realistic settings: flow concentration (channelization) through the filter, timing of pesticide application compared to other drivers, assumptions about the pesticide degradation trapped in the filter between runoff events, and seasonal presence of a shallow water table near the water body. We identify instances in which the importance commonly assigned to these factors is not supported by the mechanistic analysis, where other factors different than those proposed largely control the results of the assessments.

**249 Sensitivity analysis for an integrated avian fate and effects model**

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Ecological risk assessment models integrate information on exposure, toxicity, and life history to predict potential risk to ecological receptors. However, the underlying biological and ecological processes occur at different temporal rates and are often measured at different levels of resolution. These considerations of scale and measurement resolution influence the importance of different parameters for conclusions about risk that can be illuminated using model sensitivity analysis. We present a thorough sensitivity analysis of the MCnest model for each of these three model parameter categories (life history, exposure, and toxicity). For toxicity we include two terrestrial exposure models, the Terrestrial Residue Exposure Model (T-REX) and the Terrestrial Investigation Model

(TIM), which operate at different levels of complexity. Among life history parameters, nest and adult survival rates are the most influential parameters determining seasonal productivity. Among exposure and toxicity parameters, application rates, water solubility, and degradation rates were the most important parameters. Different sensitivity metrics conveyed different information about the effects of perturbations in model parameters and were not necessarily correlated with each other. This highlights the importance of careful thought about what metric to choose to characterize model

**250 Coupled exposure-effect models for evaluating VFS efficacy to mitigate pesticide risk to aquatic ecosystems**

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Environmental Risk Assessment (RA) of pesticides is based on the comparison of environmental exposure concentrations (EECs) with concentration thresholds that are considered protective of aquatic organisms based on laboratory toxicity testing. However, a better understanding of the implication of long-term pesticide exposure on aquatic ecosystems is missing. This understanding could be accomplished by linking exposure models and mechanistic aquatic effects models. Recent improvements in mechanistic aquatic effects models, like AQUATOX, allow for better estimates of the potential ecological effects of chemical pollutants on different food-web compartments at a daily resolution. Vegetative filter strips (VFS) models such as VFSMOD are used to simulate the influence of VFS and riparian buffers as a mitigation practice with demonstrated efficacy in reducing EECs in higher tier pesticide RA. However, the potential to predict the impact of these mitigations on aquatic organisms has yet to be studied in a coupled exposure-effects framework. In this research, we link EPA's long-term pesticide RA modeling framework (Pesticide in Water Calculator, PWC) with AQUATOX to evaluate the efficacy of VFS as a mitigation measure in order to reduce long term pesticide risk to freshwater organisms. EPA's agro-ecological scenario for Oregon-wheat with variable VFS lengths, from no VFS to 9 m, was selected at the margin of a representative river segment to simulate daily 30-yr runoff, sediment and pesticide loads at the river edge. The field loads together with daily hydrologic and climatic changes were used as daily inputs to AQUATOX parameterized as a river segment matching ecological characteristics of a representative salmonid river in Oregon. We estimated the effects of pesticide loads on the different ecosystem functional groups, and the potential VFS for mitigating observed pesticide effects over the 30-yr period. Relevant metrics describing exposure and effects are explored. Preliminary results indicate that VFS could be a useful mitigation measure to prevent pesticide-derived long-term effects on freshwater organisms, but that there is a non-linear translation from change in exposure to magnitude of effects based on the efficacy of VFS.

**251 Watershed modeling: Comparison of process-based model PRZM/SWAT with regression-based model SEAWAVE-QEX**

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Water quality monitoring data, specifically for pesticides, can represent best-available exposure profiles relevant to ecological risk assessment; however, there are challenges in synthesizing these data toward making conclusions about the nature of the potential range of risk. Some of these challenges include: data collection frequency, monitoring period duration, interpreting exposure profiles from one location to another, and monitoring system scale. To address these challenges, statistical approaches may be applied to characterize empirical trends. Additionally, process-based numerical modeling approaches can offer a different perspective on synthesis and interpretation of monitoring data. A comparison of a statistical and process-based numerical model was conducted to evaluate strengths and weaknesses of representing, synthesizing, and conclusions from monitoring data. This comparison was developed from pesticide measurements of six intensively-monitored HUC12 headwater watersheds in the Midwest. SEAWAVE-QEX is a regression model that incorporates a linear trend term, covariates accounting for seasonality, and a transformation of flow to represent a long-term pesticide trend at a specific monitoring location. PRZM/SWAT (Pesticide Root Zone Model/Soil Water Assessment Tool) is a spatially-distributed hydrologic and chemical transport numerical model that combines upland chemical and hydrologic processes from PRZM and stream flow and chemical transport processes from SWAT. The predictive quality and limitations of these two models (SEAWAVE-QEX and PRZM/SWAT) was assessed against observed, daily concentration measurements as well as hypothetical data collection frequency and timing (derived from sub-sampling the same data sets).

**252 Taxa-focused approach to standardizing use of population models in ecological risk assessment**

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In spite of widespread acceptance of the utility of population modeling and advocacy of these approaches for more ecologically relevant perspective, they are not routinely incorporated in ecological risk assessments (ERA). A systematic framework for situation-specific model development is one of the major challenges to broadly adopting population models in ERA. As risk assessors confront the multitude of species and chemicals requiring evaluation, adaptable model templates and taxa-specific guidance on model parameterization would facilitate this process. We build on previous work that created a framework and decision guide for developing population models for ERA by focusing on anuran amphibians. Anurans have a unique life cycle with varying habitat requirements and physiological processes. These species belong to the amphibian class, which is facing global population declines, due in large part to anthropogenic stressors, including pesticide effects. A stage-based matrix model broadly represents the anuran life cycle. We synthesize information from databases and literature relevant to amphibian risks. At the individual, population, and ecological levels we identify traits that influence inherent sensitivity, population vulnerability, and environmental constraints. We also outline methods of modifying a basic population model to suit specific risk assessment needs with appropriate scale and parameterization to evaluate pesticide effects. A standardized population model approach for anuran ERA offers an example method of identifying potential risks and determining long-term impacts of chemical stressors to populations across taxonomic groups.

**253 Mechanistic modeling of chemical stressors: Comparison of toxic effects on different trout species**

*C. Accolla, V. Forbes, University of Minnesota / Ecology, Evolution and Behavior*

Ecological risk assessment ought to make predictions about the likely effects of chemical contaminants on populations and ecosystems. However, current approaches often fall short because methods for estimating and integrating exposure and effects are based on overly simplistic assumptions. Mechanistic models have an essential role to ensure better predictive capacity, integrating the effects of chemicals across multiple levels of ecological organization. Furthermore, models can extrapolate both stressor impacts and life histories across species that cannot be tested in the lab. The aim of this research is to compare the impacts of a generalized stressor affecting ingestion among three trout species: the rainbow trout (*Oncorhynchus mykiss*), the brown trout (*Salmo trutta*)and the threatened greenback cutthroat trout(*Oncorhynchus clarkia stomias*). The comparison is performed at individual and population scales. To this end, we developed an individual-based model (IBM) for each trout species.

The individual description is based on Dynamic Energy Budget (DEB) theory, which describes the metabolic organization of organisms and which is a standardized theory, tested for many species in different contexts. Each species is characterized by a set of DEB parameters, as well as by different seasonal reproduction periods. At the individual level, we compare scenarios in which temperature is consistently optimal or fluctuating, while food is always unlimited. Responses to the toxicant show that the relative impact of the stressor, calculated as a percentage decrease in growth or fecundity compared to control, is the same among species and among scenarios. However, temperature fluctuations delay reproduction and reduce length and fecundity. At the population scale, we represent a more realistic environment, in which temperature and food fluctuate seasonally. Results at the individual level suggest that differences among DEB parameters do not influence model outputs. A good representation of temperature fluctuations, instead, seems to be crucial. This result is particularly relevant if corroborated at the population scale, meaning that well sampled and well parametrized species can be used as surrogates for closely related species for which data are not available.

**254 Modeling effects of stressors at population scale from observed effects at individual scale**

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Environmental stressors, such as invasive species, climate change and chemical pollution increasingly threaten ecosystems and their services. These stressors can have multiple lethal or non-lethal effects on individuals, such as increasing mortality rates, decreasing reproduction and growth rates or changes in other life-history traits. These effects are often measured at the individual scale for experimental convenience. These experiments provide essential information about stressor hazardousness and about the biological mechanisms of toxicity in exposed organisms. However, ecological management and protection generally occur at population, community or ecosystem levels. Moreover, observed effects at the individual scale cannot be taken as proxies for effects at higher scales of biological organization. Thus, there is a need to extrapolate the consequences of stressors at the population scale from those observed at the individual scale. Mechanistic modeling is an excellent tool for this purpose. In this work, we aim to compare how individual-scale effects translate to the population scale in different ecosystem types. For this purpose, we developed an individual-based model (IBM) of a well-known surrogate fish species, the fathead minnow (*Pimephales promelas*). In this model, individual fishes, whose metabolism is described with the Dynamic Energy Budget (DEB) theory, are simulated in ecosystems controlled by either food availability or predation. In addition, we simulated individual fishes that are also exposed to a toxicant. We considered scenarios in which the toxicant effects were lethal (i.e., increased vulnerability to predation for particular life stages) or non-lethal (i.e., effects on ingestion, maintenance, reproduction or growth). Results show that the intensity of effects at the population scale on fish density varies depending on which individual-level toxicant effects are considered. More importantly, results highlight that the effects on fish density strongly depend on ecosystem type such that individual effects were potentially overcompensated, compensated, partially compensated or not compensated at all. These results are particularly relevant in the context of ecological risk assessment because they underline the importance of the interplay between stressors and the density-dependent mechanisms regulating population dynamics.

**255 Setting a species-specific population model in the context of an aquatic food web model: A case example for the Topeka shiner**

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For species listed as threatened or endangered under the Endangered Species Act (ESA), higher-tier environmental risk assessments (ERAs) include assessing potential overlap of pesticide use patterns and the species' geographical range. However, potential risks to populations also depend on temporal aspects of exposure and effects as well as interactions with ecosystem-mediated effects. We developed an individual-based population model (IBM) for the Topeka shiner (*Notropis topeka*), a fish species listed as endangered, and linked it with an aquatic food web model (comprehensive aquatic systems model, CASM). The CASM was parameterized and calibrated to represent the waterbody conditions and aquatic species community in a small headwater pool in Iowa, representative of a Topeka shiner's key habitat within its geographical range. In the IBM, Topeka shiners feed on several groups in the aquatic food web according to published diet studies. Fish are simulated to grow according to a bioenergetics submodel that considers consumption, respiration and waste losses. We modelled the effects of alterations of the food web on the Topeka shiner populations by systematically reducing the available biomass of groups that the shiners consume. These alterations are generic simulations of the potential effects of pesticide exposures in agricultural landscapes around the shiner's habitat. Potential pesticide effects on the food web depend on the compound, magnitude, timing and duration of exposure, and are simulated in the model by applying daily concentrations of a pesticide in the simulated waterbody which can be derived from field measurements or fate models. The linked models provide a platform for the assessment of potential direct and food-web mediated indirect effects of stressors such as pesticides on species populations.

## Human Health and Ecological Risk Assessment of Per- and Polyfluoroalkyl Substances

**256 Application of revised methodologies for default guideline value derivations: PFOS in freshwater**

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As part of a review of the Australian and New Zealand guidelines for fresh and marine waters, new default guideline values (DGV)s have been derived for a range of priority chemicals. A reassessment of the scientific underpinnings of DGV derivation was also undertaken paying particular attention to the acute and chronic definitions for tested species, and guidance on the use of species sensitivity distributions (SSDs) including the assessment of modality, determining the reliability of the derived DGVs, preferred toxicity endpoints and the use of multigenerational tests (Batley et al., 2018; Warne et al., 2018). PFOS was one of the chemicals having a new DGV, derived by Golder. This presentation focuses on subsequent changes to the derivation methodology for such bioaccumulating toxicants with assessment of modality where mode of action is uncertain or unknown. The preferred DGV derivation method is to use SSDs. For compounds such as PFOS which bioaccumulate and biomagnify, the 99% species protection value (PC99), rather than the PC95, is recommended for slightly to moderately disturbed ecosystems. The Canadian DGV of 6.8 µg/L is a PC95 value from an SSD. The EU concluded that there were insufficient chronic data to derive an annual average environmental quality standard (EQS) using an SSD, so applied an assessment factor of 10 to the lowest NOEC to obtain a value of 0.23 µg/L. However, to be protective of human health, an EQS of 0.00065 µg/L was chosen for inland surface waters. In the Netherlands, RIVM applied an assessment factor of 100 to obtain a maximum permissible concentration of 0.023 µg/L, but for secondary poisoning derived a value of 0.0026 µg/L. In Australia, eighteen chronic data from five taxonomic groups, including a sensitive multigenerational 180-d growth test on zebrafish, were used in an SSD plot that spanned an unusual seven orders of magnitude. The very low slope of the tail of the SSD resulted in the 99% protection value being extrapolated to 0.00023 µg/L. There was a separation of sensitive animal data and less sensitive plant data, implying bimodality. The animal data spanned five orders of magnitude with a PC99 value of 0.039 µg/L. Applying a newly developed weight of evidence screening for modality assessment (Warne et al., 2018), it was concluded that the data were bimodal and that the proposed DGV should be based solely on animal data. This, and other significant considerations for the PFOS DGV derivation will be discussed.

**257 Bioaccumulation of Per- and Polyfluoroalkyl Substances (PFASs) from Exposure to Aqueous Firefighting Foam (AFFF) Mixtures in Mouse and Human Serum**

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Per- and polyfluoroalkyl substances (PFASs) are a class of highly fluorinated synthetic organic compounds that have become ubiquitous global pollutants due to their remarkable environmental persistence. Recently, PFASs have entered the national spotlight due to widespread contamination of U.S. drinking water supplies, raising serious concerns about their bioaccumulation potential and health effects. Aqueous firefighting foams (AFFFs) used to combat hydrocarbon fuel-based fires are a significant source of PFASs in potable water. More information on bioaccumulation, biotransformation, and biological effects of AFFF-associated PFASs is rapidly needed to understand health risks posed by widespread, long-term AFFF contamination of drinking water. Here, samples of serum from mice dosed with a commercial AFFF formulation, which is a mixture of hundreds of PFASs, were analyzed via liquid chromatography with quadrupole time-of-flight-mass spectrometry (LC-QToF-MS) to screen for PFASs and their transformation products. Serum samples from 200 humans in the Fountain-Widefield-Security communities of Colorado, where widespread AFFF contamination of drinking water has occurred, were also collected and analyzed by LC-QToF-MS. A custom high-resolution mass spectral library and extensive extracted ion chromatogram (XIC) list developed at Colorado School of Mines were used to screen samples for novel fluorinated compounds. To evaluate bioaccumulation potential, the composition of PFASs in mouse serum was compared to the commercial formulation used in dosing to determine which PFASs were relatively enriched in the body and which were depleted. Preliminary results suggest that some of the PFASs that appear to be most bioaccumulative, including unsaturated, chlorinated, and oxygen-containing derivatives of C7-C11 perfluoroalkyl sulfonates, are novel PFASs for which little to no biomonitoring or toxicological data is currently available.

**258 Dietary Exposure of Japanese Quail (*Coturnix japonica*) to Perfluorooctane Sulfonate: Effects on Reproduction and Chick Survivability and Growth**

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Aqueous film forming foam (AFFF) has been used by the Department of Defense for over 40 years for fire-training and emergency response activities. As a result of these activities, ground water, surface water and biota in the vicinity of relevant military installations have become contaminated with perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and other poly- and perfluoroalkyl substances (PFASs). As part of an effort to develop avian ecotoxicity information for compounds associated with AFFF, the effects of dietary PFOS on reproduction and chick survivability and growth in an avian species recognized as a surrogate for wild avian species, the Japanese quail (*Coturnix japonica*), were determined. Day-old Japanese quail were administered PFOS at analyzed dietary concentrations of 0, 2.1, 4.0, 8.6, 14 or 18 µg/g feed for a total of 140 days. At four weeks of age, 16 male/female pairs were assigned to each treatment group. At 10 weeks of age, females began laying eggs. For the next 10 weeks, eggs were collected daily, set weekly and incubated for 18 days. On the day of hatch, hatchlings were counted, weighed and placed in a brooder battery on clean feed for two weeks. Unhatched eggs were opened to determine the stage of development. At the end of the second week, 10 chicks from each treatment were euthanized and sampled for blood and liver. Liver and pooled serum samples were analyzed for PFOS. At the end of the 10th week of egg laying, adult birds were euthanized and sampled for blood and liver for PFOS analysis and liver, kidneys, lung and thyroid glands for pathology. At the end of the trial, body weights of adult quail fed feed containing PFOS were significantly less compared to control body weights. Egg production of hens fed PFOS was significantly less in all treatment groups except the 14 µg/g feed group compared to controls. Hatchability of eggs was significantly less in the 8.6, 14 and 18 µg PFOS/g feed groups compared to hatchability in the control group. Survivability of chicks in the PFOS treatment groups through the first week of age was generally less compared to the control group whereas body weights of two-week-old quail were not significantly different across treatments. Results of this study suggest that dietary exposure of Japanese quail to PFOS has an adverse effect on reproduction and chick survivability at concentrations ranging from 2.1 to 18 µg/g feed, but chick growth is unaffected.

**259 Observed distribution of PFOS in biota in a tropical environment**

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Australian Department of Defence is undertaking a nationwide investigation program to assess the nature, extent and impact of PFAS contamination from legacy AFFF use on Defence sites. Where the abiotic data indicated a plausible pathway or elevated risk to human health or ecology, sampling and testing of biota was also conducted. This paper presents key observations from biota testing conducted across aquatic, riparian and terrestrial settings related to two RAAF Bases in the Northern Territory of Australia. The sites are in tropical climates and biota included a broad range of native and introduced species. Combined across the two sites, over 50 vegetation, 120 terrestrial vertebrates (including mammals, reptiles, amphibians and birds), 50 invertebrates and 700 fish samples were tested. Analysis indicated differing patterns of accumulation in animals, with low concentrations in green ants (less than 4 mg/kg) and high levels in reptiles (up to 5,400 mg/kg) in the same area. Sampling across wet and dry seasons also showed accumulation and elimination at different rates in fish species, with large long-lived species showing little seasonal trend, but an increase with age, and smaller species showing a distinct seasonal fluctuation independent of age. The results of the testing have been used in ecological risk assessments to validate food web relationships, identify the most significant pathways and provide a baseline for future reference.

**260 An evaluation of the potential impact of perfluorooctane sulfonate (PFOS) to mink and otters: Current perspectives**

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Perfluoroalkyl acids (PFAAs) are synthetic fluorinated organic compounds that can be released to the environment during manufacturing processes, from commercial products and applications, or indirectly via oxidation of precursor molecules containing perfluoroalkyl chains. While PFAAs have received increased attention in monitoring programs, the assessment of the potential risks PFAAs pose to wildlife is still being investigated. Few ecologically relevant toxicity studies have been conducted with wildlife exposed to PFAAs and this is particularly true for mammalian species. To address this issue, we used the USEPA Great Lakes Initiative (GLI) to derive protective values for perfluorooctane sulfonate (PFOS) in mink, a sentinel wildlife species. Benchmark dietary and liver PFOS concentrations were determined using data from a multigenerational rat study and a mink pharmacokinetic study. Bioaccumulation and biomagnification factors were used to extrapolate toxic doses water value (WV) for mink and otters. While the GLI approach focuses on food web extrapolations to water, the literature indicates that sediments may also be an important vector of PFOS to aquatic organisms and upper trophic level predators. As a result, the GLI may produce overly protective water values. A simple food web model was used to estimate the significance of water and sediments as sources of PFOS to mink. The results from this analysis indicated that the sediments are a source of PFOS to mink and should be taken into consideration when setting water values. Finally, these wildlife values and tissue benchmarks are discussed relative to current and historical PFOS concentrations measured surface water and mink.

**261 Firefighters' accidental exposures to Aqueous Film Forming Foams- A case study**

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Per and polyfluoroalkyl substances (PFASs) are a class of compounds extensively used in food packaging, commercial goods, and aqueous film forming foams (AFFF) used to extinguish fires. Some of these compounds, such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), are highly persistent in the environment and biota, and can cause adverse health effects, such as endocrine disruption, immunological suppression, and cancer. Concerns about AFFFs contaminating ground water have led to restrictions on their use. Additionally, concerns regarding occupational exposures of firefighters to PFAS contained in AFFFs are mounting. We report a case study involving 3 professional firefighters who were accidentally splashed with AFFF in 2015. Serum samples submitted by their physician were analysed for PFAS using targeted and non-targeted analytical approaches using online-SPE-HPLC-MS/MS system, offline SPE and UHPLC-MS/MS, and UHPLC-QTOF-MS. Our targeted analysis showed that these firefighters had PFOS, PFOA and PFHxS above the $90^{th}$ percentile of NHANES (males, 2013-14). Additionally, several markers of historical and current use AFFF including PFOS, 6:2 FtS and short-chain carboxylates (e.g. PFHxA) were elevated in those firefighters compared to other study populations. Using our in-house PFAS database with ~400 PFAS compounds, suspect screening analysis (SSA) also confirmed those compounds with high intensities. However, we did not detect any novel AFFF formulations and transformation products that we identified in wastewater samples previously. Unknown identification using chemical formula generator, polyfluorine mass defect filter, and fragmentation studies are underway. The views expressed herein are those of the authors and do not necessarily reflect those of the California Department of Toxic Substances Control.

**262 Development of Risk Based Screening Criteria for Poly and Perfluoroalkyl Substances: Recommendations, Challenges, and Research Gaps**

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Poly and perfluoroalkyl substances (PFASs) have been identified as chemicals of concern in agricultural soils, biosolids-amended soil, and irrigation water due to their toxicity, persistence, and bioaccumulation potential. The agricultural use of PFAS-contaminated irrigation water, municipal and industrial biosolids, and municipal compost can all contribute to the exposure of PFASs to food crops. Soil irrigated with contaminated water may become contaminated over time resulting in additional exposure pathways to food crops. The unintended uptake and accumulation of PFASs in edible crop plants is an important and continuing concern for protecting human health. In particular, there is an urgent need for regulatory agencies to accurately understand accumulation in the edible portion of food crops as these foods are often consumed fresh or with minimal processing. Recognition of PFASs in food as an important contributor to human exposure, as well as the identification of PFAS-impacted groundwater and soils in areas with agricultural activities, has resulted in several studies on the uptake of PFASs into crops. Using available crop-specific bioaccumulation factors (BAFs) and consumption rates for food crops typically grown in urban gardens and farms, as well as best available or derived human health reference doses, risk based concentrations (RBCs) for PFASs in contaminated soil and irrigation water were determined for adults and children. The RBCs for six perfluoroalkyl carboxylic acids and three perfluoroalkyl sulfonic acids in soil and water are compared to other risk-based pathways and available health advisories or criteria. Preliminary results indicate the RBCs for perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) in soil are lower than available EPA or state residential soil screening levels; whereas, the RBCs for PFOS and PFOA in water are generally less conservative than the current health advisory for drinking water of 70 ng/L. Typically, non-root vegetables (e.g., lettuce) as compared to root vegetables, fruit, wheat, and corn have the lowest RBCs for contaminated soil and water; however, the RBCs are highly site specific due to varying soil organic carbon content, growing season, and consumption. The variability of the RBCs and model parameter sensitivity (e.g., BAFs) are evaluated. The BAFs vary considerably between crop types, experimental conditions, and compounds. Key research needs are discussed and data gaps are identified.

**263 Monitoring and Analysis of Poly and Perfluorinated Alkyl Substances in the European Honey Bee at Williamtown, New South Wales, Australia**

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Poly and perfluorinated alkyl substances (PFAS) contaminate the environment around the Williamtown Royal Australian Air Force site, NSW, Australia. The PFAS compounds are sourced from the Aqueous Film Forming Foam, historically used for defence force firefighting activities. Water soluble and persistent, PFAS have been detected in the ground and surface water, sediment, soil, and aquatic and terrestrial biota both on and off the site. Concerns regarding exposure and risk are significant with sensitive down-gradient receptors and local hydrogeological aspects that conflate exposure. Bees were selected as the test subject due to their foraging nature and range, link to human receptors from honey consumption and key pollination role. To our knowledge, analysis and investigation of PFAS accumulation in bees and honey has not been investigated in Australia to date. For one year, we monitored bee (*Apis mellifera*) tissue and honey from four hives situated within foraging range (2.5-8 km) of the perimeter of the site that includes the NSW EPA Special Investigation area, and one non-contaminated control hive. Composited bee tissue (n =

20) and honey (n = 20) was tested for PFAS compounds using an isotope dilution method combined with Liquid Chromatography Tandem Mass Spectrometry adapted for bee and honey matrix analysis. Our results indicated that PFAS concentrations, in particular perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), in bee tissue and honey were equal to test results from our control site (< 0.003 mg $kg^{-1}$). Quality control work indicated acceptable spike recovery, accuracy and precision. The PFAS control spike sample recoveries ranged from 70-138% (average 103%), honey matrix PFAS spike recoveries ranged from 77-125% (average 100%). Isotopically labelled internal standard recoveries ranged from 60-80% for PFOA and PFOS. The screening tolerable daily intake value for food consumption in Australia is less than 0.02 and 0.16 µg/kg/week for PFOS / PFHxS and PFOA, respectively (FSANZ, 2016). This preliminary study showed no evidence of PFAS uptake in bees collected within a foraging range of a significantly contaminated location. The monitoring indicated that human exposure from consumption of honey from the hives sampled was not a concern. Potential uptake pathways, bee foraging behaviour and PFAS availability were evaluated to interpret these findings.

## Persistence and Biodegradability Assessment

**264 Biodegradation screening methods: Limitations and areas for improvement**

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For down the drain disposed chemicals, the OECD 301 Ready Biodegradation series of tests are widely used by scientists and regulators to assess aerobic biodegradability typically using inocula from activated sludge wastewater treatment plants. These tests can be useful screening tools as they are well prescribed, use indirect measures of biodegradation by following carbon or oxygen, and are relatively inexpensive and easy to perform. However, the usefulness of these studies is limited due to the conservative nature of the tests. These tests employ high test chemical concentrations and low levels of inocula that are not realistic when compared to actual environmental conditions. The unrealistically high test chemical concentrations can lead to issues with solubility and microbial inhibition under the test conditions. The low levels of inocula limit the microbial diversity which is unrealistic compared to actual environmental compartments and also leads to extended time periods needed for the microbes present in the study to adapt to the test chemical as the only food source and for the microbial population to increase to a sufficient level to degrade the high concentration of test chemical. These conservative test conditions are further confounded by the stringent pass criteria and short test duration (28 d). This presentation will use case studies of different chemicals to highlight limitations of the OECD 301 test method and propose potential areas for improvement. The first case study will explore issues related to chemical solubility and microbial inhibition and the impact of modifying the test design to overcome these issues. The second case study will explore the impact of chemical bioavailability and mass transfer limitations and discuss the importance of test extension to overcome these issues. The final case study will evaluate microbial adaptation both in the field and in a lab setting and highlight the importance of evaluating environmentally relevant adaptation in an assessment of biodegradability.

**265 Fate and Remediation of an Extremely Recalcitrant Emerging Contaminant: 1,2,3-Trichloropropane (TCP)**

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1,2,3-Trichloropropane (TCP) is an emerging contaminant of exceptional concern because it is extremely toxic (the California State Water Resources Control Board recently adopted an MCL of 5 ppt), wide-spread due to a variety of industrial and agricultural uses, mobile in contaminated groundwater, and extremely persistent. The overall persistence of TCP was apparent from early lab and field studies that found essentially no evidence for degradation by biological or chemical pathways. More recent and intensive studies have identified systems that degrade TCP by hydrolysis, oxidation, and/or reduction, but all of these systems involve highly optimized or extreme conditions, and the resulting degradation kinetics are still slow relative to many contaminants. The reactivity of TCP is similar to that of other lesser chlorinated aliphatic hydrocarbons (LCHCs), which have only one halogen substituent per carbon (e.g., 1,2-dichloroethane). That makes these compounds very poor targets for dichlorination by hydrogenolysis (which often is the preferred route of microbial dehalogenation). However, once TCP undergoes reductive elimination (often the preferred abiotic pathway), the resulting intermediate (allyl chloride) is readily reduced to fully dechlorinated products. Thus, while TCP degradation is hard to initiate by reduction, problematic intermediates do not accumulate. We have shown this with a variety of abiotic reductants (esp. zerovalent zinc), modeled the kinetics, and explained the results using molecular modeling. The implications generally are not promising for biodegradation of TCP, but they are consistent with the various, recent reports of TCP degradation by a few microbial systems. Because data on the degradation of TCP are so sparse, models that predict (bio)degradation are not well calibrated for this or other LCHCs, so predictions of their persistence are not very reliable.

**266 Kinetics of Human Pharmaceutical Conjugates and the Impact of Transformation, Deconjugation, and Sorption on Persistence in Wastewater Bioreactors**

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The purpose of this study was to address the knowledge gap of simultaneous in vitro transformation of pharmaceuticals and their metabolite conjugates using real wastewater, and to corroborate the results seen in our previous field study. Wastewater parameters such as components of primary or secondary wastewater, addition of air, and/or waste activated sludge (WAS) could influence the kinetics of removal or transformation. Under a realistic wastewater treatment plant HRT (< 2h), both acetaminophen and its sulfate were rapidly degraded (>99%). There was evidence for in vitro sulfation of propranolol, with concurrent removal of propranolol; and also deconjugation of N-acetylsulfamethoxazole and sulfamethoxazole glucuronide contributing to the increase of the parent sulfamethoxazole. Thyroxine was resistant to degradation across all treatments while thyroxine glucuronide was shown to be rapidly deconjugated (>90% in < 2h). Suspended solids analysis at the 0h and 24h timepoints revealed that in absence of WAS, sorption is another major mechanism of removal for acetaminophen, propranolol, sulfamethoxazole, and thyroxine. However, in the presence of WAS, suspended solids concentrations decreased for all analytes by the 24h timepoint. These results indicate that both conjugation and back-transformation are compound specific and dependent on parameters such as HRT, addition of WAS, and suspended solids levels.

**267 Influence of structure and nanoporosity of sediment organic matter on mineralization of benzo(a)pyrene by hydrogen peroxide**

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Benzo[a]pyrene (BaP) in sediments is persistent, bioaccumulation, genetically toxic, and carcinogenic to organisms. We here investigated how the mineralization of 7-$^{14}$C-benzo[a]pyrene by $H_2O_2$ treatment in aged sediments from the Pearl River Estuary to South China Sea is influenced by the structure, fractions, and nanoporosity of sedimentary organic carbon (OC) by using mineralization bottles and liquid scintillation instrument. Unstable OC (USOC), stable OC (SOC), mineral-protected OC (MOC), and chemically resistant OC (ROC) was fractionated in the sediments. The structure and nanoporosity of the ROC fractions were characterized by NMR and CO2 adsorption techniques, respectively. It was found that BaP mineralization ratios and USOC percentages gradually decreased, but ROC percentages and aliphatic contents gradually increased from the estuary samples to the offshore marine samples. A first-order, two compartment kinetics model described the mineralization process very well ($R^2$ =0.990). The mineralization ratios of BaP at 408 h in the sediments showed a significant positive correlation with the USOC contents ($R^2$=0.847, $p$< 0.05), and a negative correlation with the ROC contents ($R^2$=0.780, $p$=0.06), suggesting that the ROC fractions can protect BaP from the oxidation. Significant and negative correlations among the mineralization ratios of BaP, kinetics parameters, and the aliphatic-C, alkyl-C, and (CH2)n-C structures, and nanopore volumes (Vo) suggested that the aliphatic moieties and nanooposity play a crucial role in protecting BaP from being mineralized by the hydroxyl radicals. This study indicated that the important role of the USOC, ROC, and aliphatic moieties and the nanoporosity of ROC in the mineralization of BaP in sediments during $H_2O_2$ treatment. Keywords: 7-$^{14}$C-benzo[a]pyrene; mineralization; hydrogen peroxide, recalcitrant OC; aliphatic carbon, nanooporosity

**268 Recalcitrant Petroleum Spills: Biodegradation and Metabolites**

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Although petroleum spills are often considered highly susceptible to processes that facilitate natural attenuation it is not unusual to come across a mature release site with levels of petroleum hydrocarbons that cause regulatory concern. Recent interest in high “TPH” levels in groundwater associated with some petroleum sites has fueled a debate about petroleum hydrocarbon metabolites (partial breakdown products with much higher water solubility than parent hydrocarbons). The debate initially focused on the potential toxicity of weathered versus fresh petroleum hydrocarbon mixtures but it since has become clear that a satisfactory resolution of this issue will also require a better understanding of what happens with petroleum hydrocarbons after their release into the environment. Thus, a literature review on hydrocarbon biodegradation that complements and supports the Petroleum Metabolites Literature Review and Assessment Framework published in 2016 by the San Francisco Bay Regional Water Quality Control Board was conducted and part of that is being incorporated into the forthcoming ITRC guidance on TPH Risk. The fact that at many sites petroleum hydrocarbon mixtures appear to degrade with astonishing ease compared to typical releases of halogenated chemicals, for instance, has given rise to “reverse-engineered” theories about biodegradation. Such theories match observations at simple sites but tend to misapply bits of chemistry and thus fail to provide insight into complex or recalcitrant sites. The latter tend to have large anaerobic portions with a vastly greater microbial and chemical complexity than fully aerobic sites. Successful evaluation and management of slowly degrading petroleum sites requires the understanding of anaerobic biodegradation including basic chemistry, biochemistry and microbiology concepts as well as the ever-expanding options for the investigation of petroleum biodegradation.

**269 Marine biodegradation screening testing version 2.0**

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Exponential growth of the human population has been accompanied by increased manufacture and use of chemical compounds. To classify the fate and behaviour of these compound in the environment, a series of international standardised biodegradation screening tests (BSTs) were developed over 25 years ago. In recent years, regulatory emphasis (e.g. REACH) has shifted from measuring biodegradation towards prioritisations based on chemical persistence. In their current guise, BSTs are ineffective as screens for persistence. They are prone to high levels of variation and produce a large number of fails, many of which can be considered false negatives, wherby a chemical fails a test not because of its recalcitrance, but rather because the test itself has failed. Based on previous regulatory recommendations and research conducted, two key methodological modifications to potentially improve the marine BST (OECD 306) were high lighted: (i) increasing bacterial cell concentrations to better represent the bacterial diversity inherent in the sampled environments; and (ii) increasing test durations to investigate extended lag phases observed in marine assessments. During 2016-2018 an improved marine BST protocol incorporating both increased cell concentrations and test duration was assessed using a set of reference chemicals in a multi-institutional ring test across 13 laboratories in the UK, Norway, Germany, Italy, Canada, USA and Japan. In comparison to the standard marine BST, the improved marine BST was more reliable and less variable at characterising the environmental persistence of the reference compounds. Extending the test durations beyond 28 days allowed for a more reliable characterisation of environmental persistence. There was a positive relationship between total cell counts and biodegradation potential. However, the greatest rates and extents of degradation were not necessarily observed at the laboratories with the highest cell concentrations, suggesting that cell concentration is not the only factor influencing the degradation potential of an environmental sample. Preliminary microbial analysis suggests that the composition of the sampled environment may have a greater influence on biodegradation test outcome than diversity.

**270 Influence of microbial adaptation in persistency testing**

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Regulatory determination of the persistency of organic chemicals is often based on the results of OECD ready biodegradability tests (RBTs). These tests, however, suffer from several problems that lead to a high variability of the results and, hence, to difficulties in their interpretation. The origin and history of the inocula is one of the major causes of that variability. Nowadays, it is evident that results of RBTs change over time as microbial populations apparently adapt to metabolise previously persistent chemicals. Several studies also show an improvement of the biodegradation rates even after a short period of pre-exposure to the tested chemical. As such, there is a need to assess the influence of this process on biodegradability tests. In this study we assessed the extent of the inocula origin influence on the outcome of biodegradability testing. For this, we compared the biodegradation capacity of five different wastewater treatment plants across the Netherlands to eliminate carbamazepine, n-methylpiperazine, metformin, diclofenac and 4-chloroaniline. Most of these products can be considered as emerging pollutants and are environmentally persistent. In a second time, we used chemostat systems to expose activated sludge microbial communities to carbamazepine, n-methylpiperazine, metformin and 4-chloroaniline for several months under defined conditions. In both experiment, the biodegradation capacities of the inocula were assessed in biodegradation testing, following the OECD 310 guideline, and changes in community structure were followed by Illumina amplicon sequencing in time. Removal of tested chemicals and their transformation products were determined by GC-FID and LC-MS/MS. Results of these experiments show difference of biodegradation capacity between the tested inocula. Long term exposure of a microbial community to n-methylpiperazine in chemostat leads to an enhanced biodegradation of this compound. These results will allow a better understanding of the relationship between microbial adaptation and biodegradation performance. Moreover, microbial communities exposed to metformin were able to degrade its known persistent transformation product, guanylurea, which is persistent in fresh water. Ultimately they will also allow more realistic predictions of their biodegradation in the environment compared to those obtained using standard testing protocols.

**271 Towards an improved understanding of persistence in the 21st century**

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There is increasing concern that progress in the scientific understanding of factors which influence the outcome of chemical persistence (P) assessments (particularly biodegradation) are not fully recognised in existing regulations and environmental risk assessments. As all biodegradation tests have their limitations, which are accentuated for 'difficult to test' substances, failure to recognise these can potentially lead to many chemicals being incorrectly labelled as 'P' persistent. Many of the shortcomings of existing biodegradation studies have been recognised and discussed in a series of ECETOC reports and workshops. Key recommendations from these have been used to help develop and formulate a series of CEFIC LRI persistence assessment-related research projects, the emphasis being to address the key issues and propose methods to mitigate the existing limitations. However, many of the recent developments recommended in the multi stakeholder workshops and related research projects have not been incorporated into existing regulatory frameworks for environmental hazard and risk assessment. If the current situation is not challenged there are concerns that this will lead to an overestimation and over-classification of the hazards and risks posed by a wide range of chemicals, potentially leading to a significant amount of unnecessary testing and restrictions on advantageous chemical use. Any proposal to modify guidance needs to be based on sound science that re-examines the fundamentals of biodegradation and what existing test methods provide. Therefore, to initiate this process an overview of some of the key elements and messages coming from recent research initiatives, how these interrelate and redress some of the previously identified P assessment needs will be presented and discussed at a 'persistence' workshop to be held in Helsinki on the 27$^{th}$ September 2018. The objective being to assess how recent scientific and technological developments are given appropriate consideration by regulatory agencies and the wider community in chemical persistence assessments. This presentation will provide a summary of the key messages from the workshop regarding how best to utilise these new developments and the next steps required to start a journey to take the persistence assessment of chemicals into the 21$^{st}$ Century.

## Pesticide Runoff Pollution: Challenges and Opportunities in Prediction, Prevention and Management

**272 Adequacy and uncertainty in models and scenarios developed to estimate pesticide wash-off and runoff in residential settings**

*W.M. Williams, Waterborne Environmental, Inc.; Y. Luo, California Department of Pesticide Regulation; M.F. Winchell, Stone Environmental, Inc. / Environmental Systems Modeling*

A variety of pesticide products, including insecticides, herbicides, and fungicides, are used by homeowners and licensed Pest Control Operatiors to eradicate or deter pests on lawns and ornamentals, vegetable gardens, homes, and other structures. Pesticide detections in urban streams have resulted in an increase in research over the past decade to understand the potential for pesticide washoff to occur from various pervious and impervious application sites. Regulatory agencies, chemical registrants, and other researchers have employed a variety of approaches to model pesticide runoff associated with residential uses. This presentation compares the underlying prinicipals in several models and scenarios that have been adopted or proposed for pesticide registration review and risk assessment by regulatory agencies in the U.S. and the E.U.; and evaluates their appropriateness for risk assessment for various categories of outdoor residential-use pesticide products. The models/scenarios include the Home and Garden scenario developed for the HardSPEC model by the Food and Environment Research Agency in the U.K., residential scenarios developed for PRZM5 by the U.S. Environmental Protection Agency, and adapting urban stormwater management models including the Stormwater Management Model (SWMM5) and the P8 Urban Catchment Model.

**273 Comparison of Model Simulated and Measured Pesticide Runoff Losses from Residential Landscapes**

*M.F. Winchell, Stone Environmental, Inc. / Environmental Systems Modeling; R.L. Jones, Bayer CropScience / Environmental Safety*

Mechanistic models of pesticide fate and exposure have been developed and applied for regulatory purposes for use in the US and abroad for several decades. Models such as the Pesticide Root Zone Model (PRZM), which was originally developed to simulate processes in agricultural settings, have been extended for use in residential and other urban environments (e.g. USEPA residential PRZM scenario, CAresidentialRLF.scn). More recently, urban hydrology models, such as the Stormwater Management Model (SWMM), have been adapted to simulate pesticide transport processes from heterogeneous residential landscapes that include mixtures of pervious and impervious surfaces. The runoff transport algorithms in PRZM, SWMM, and other mechanistic and empirical models are highly dependent upon the parameterization of the surface hydrologic processes. Consequently, these can have significant impacts on the amount of off-site pesticide transport and ultimately, the predicted concentrations in receiving waters. In order to generate confidence in the predictions of pesticide fate and transport models applied to residential environments, the model predictions must be compared with and validated against field measurements. This presentation will compare measured pesticide runoff mass losses from a field study conducted in controlled outdoor residential house lots in California with estimated pesticide runoff mass losses from both USEPA PRZM scenario simulations, that have been used in recent ecological risk assessment for both pyrethroids and organophosphate pesticides, as well as from an adaptation of the SWMM model. These comparisons will provide information on the uncertainty inherent in the mechanistic models currently being used in regulatory decision making for residential pesticide uses, and offer some insight regarding the potential need to revisit model parameterization assumptions and realistic scenario development to better reflect observations from available field data.

**274 Environmental Risk Assessment of Outdoor Use Insecticides in the European Union: Current Approaches, Strengths and Weaknesses**

*P. Mason, SC Johnson EurAFNE Limited / GSARA*

Within the European Union (EU), insecticides used in outdoor residential situations are regulated under the Biocidal Products Regulation (BPR) (Regulation (EU) 528/2012), and the plant protection products regulation (PPPR) (Regulation EC 1107/2009). In the case of the BPR, guidance on estimating environmental emissions for insecticides (Product Type 18) is provided in the OECD Emission Scenario Document (2008), while subsequent technical decisions are documented in Technical Agreements on Biocides (TAB). This presentation will provide an up-to-date overview of the scenarios used for estimating emissions to relevant environmental compartments for different forms of outdoor use insecticide product under the BPR. This will include a review of assumed use patterns and treatment areas. The work will also highlight key uncertainties in the emission estimation frameworks, including the challenges associated with assessing highly localised soil exposure, as well as 'scaling-up' wastewater emissions to the catchment level. An update on latest approaches for calculating Predicted Environmental Concentrations in soil, surface water and sediment, as described in the recently updated Guidance on the

Biocidal Products Regulation Volume IV Environment (ECHA, 2017) will be provided, highlighting some of the regulatory issues that are currently under discussion in the EU.

**275 California's collaborative aquatic risk mitigation for fipronil in urban waterways and the important role of urban runoff modeling**

*N. Singhasemanon, Y. Luo, R. Budd, M. Ensminger, California Department of Pesticide Regulation / Environmental Monitoring Program*

Over the past several years, the California Department of Pesticide Regulation (CDPR) has been engaged in a collaborative problem-solving effort with pesticide and water quality stakeholders to address elevated concentrations of the insecticide active ingredient fipronil in urban runoff and receiving waters. California monitoring data from 2008–2016 indicated that fipronil was being detected at a relative high frequency (~49%) in both residential wet and dry weather runoff as well as in urban area creeks. Furthermore, fipronil concentrations exceeded USEPA's chronic and acute aquatic life benchmarks for invertebrates at 48% and 15%, respectively. In 2016, CDPR made the risk management decision to pursue mitigation to address this surface water quality concern using its regulatory authority and scientific data-driven approach. CDPR collaborated with stakeholder groups including product registrants, University of California researchers, and professional pesticide applicators to: 1) better identify fipronil sources and pathways to surface water, 2) determine the amount of load reduction necessary for successful mitigation, and 3) evaluate various application practices that could lead to practical use restrictions beyond existing product labels while maintaining product efficacy. In support of these objectives, CDPR relied heavily on data from field studies and output from urban runoff modeling. For the latter, CDPR utilized USEPA's PRZM-VVWM with various modifications to achieve a more realistic and reliable simulation of California residential runoff scenarios. This integration of predictive modeling into CDPR's continuous evaluation process (as well as initial product registration evaluation) represents a more transparent, scientifically-robust, and effective approach to protect California surface water from the use of pesticides.

**276 Management options for reducing pesticide concentrations in urban runoff**

*R. Budd, Y. Luo, M. Ensminger, N. Singhasemanon, California Department of Pesticide Regulation / Environmental Monitoring Program*

The California Department of Pesticide Regulation (CDPR) is responsible for preventing significant deleterious effects of pesticides in the environment. Pesticides are widely recognized as contaminants of concern in surface waters receiving urban runoff inputs. CDPR's Environmental Monitoring Branch frequently detects fipronil and pyrethroid insecticides at concentrations that are potentially toxic to aquatic species such that mitigation is necessary. In contrast to agricultural runoff, which has more physical treatment options available for pesticide removal, mitigation strategies addressing urban runoff frequently rely on changes to application-related practices. To address ecological concerns surrounding fipronil and pyrethroids from urban runoff, CDPR developed regulations and label use restrictions for products containing pesticides of concern. CDPR worked with several collaborators including product registrants, university researchers, and professional applicators on a collaborative approach to create application restrictions that are both effective at reducing pesticide transport off urban landscapes and adoptable by the regulated community. The additional restrictions were science driven, utilizing a predictive model to estimate runoff pesticide concentrations resulting from proposed restrictions. The modelled runoff concentrations resulting from the restricted pesticide application scenarios were supported by empirical evidence obtained during experimental field trials.

**277 Biochar amended filter socks: The future of pesticide runoff mitigation?**

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Pesticide losses in surface runoff from agricultural fields following storm events is a significant water quality problem. Long-term watershed research has demonstrated that most herbicide transport, along with the highest concentrations, occurs in the first few runoff events following application. Edge-of-field runoff mitigation strategies are necessary to reduce the impact of agricultural non-point source pollution on downstream aquatic receiving systems. Traditionally used for erosion control, filter socks are mesh cloth tubes that can be filled with various sorbent materials. Biochar (derived from pyrolysis of hardwoods) has been demonstrated in the laboratory to more rapidly sorb and remove herbicides from water than other fill materials such as steel slag and tire chips. Therefore, the objective of this research was to determine if filter socks filled with woodchips or a mixture of woodchips and biochar would decrease herbicide concentrations associated with storm runoff from corn fields within the South Fork of the Iowa River during the 2017 growing season. Unfavorable climatic conditions resulting in the driest June and July on record yielded only four small runoff events during the sampling season. Results demonstrated a similar decrease in atrazine, atrazine metabolites, and isoxaflutole concentrations by both treatments (woodchips; woodchips + biochar). Only cyprosulfamide concentrations were significantly reduced by woodchips ($p = 0.0454$) and woodchips and biochar ($p = 0.0028$) compared to control (pond) concentrations. While promise is shown for using biochar-amended filter socks as an edge-of-field management practice, further evaluation must be conducted on different pesticide classes and geographical locations.

**278 Constructed wetland treatment of urban pesticides in surface water**

*Z.M. Cryder, J. Gan, University of California, Riverside / Environmental Sciences*

Pesticides are often utilized in urban environments for the control of various insect pests. Urban pesticides can be applied outdoors for structural and landscape pest control as well as indoors for household pests and veterinary flea treatments. Fipronil and synthetic pyrethroids are very commonly used for such purposes. Fipronil and its degradation products (collectively: fiproles) and synthetic pyrethroids such as bifenthrin, permethrin, and cyfluthrin are transported offsite in stormwater runoff and in wastewater following outdoor and indoor applications, respectively. Stormwater is seldom treated prior to being deposited into surface water, carrying with it all the pesticide residues washed away during runoff events. Wastewater undergoes extensive treatment, but pesticide concentrations remain sufficiently high in wastewater effluent to elicit adverse effects from sensitive aquatic organisms upon deposition into surface water. Constructed wetlands offer a promising strategy for removal of fiproles and pyrethroids from surface water. The present study focused on removal of fipronil, fipronil desulfinyl, fipronil sulfide, fipronil sulfone, bifenthrin, and cyfluthrin in a traditional constructed wetland containing planted emergent macrophytes and in a unit process open water wetland which encourages photolytic breakdown of contaminants. Influent and effluent water samples were collected monthly from both wetlands to assess the removal of the compounds of interest as a result of the physical, chemical, and biological processes occurring in the treatment systems. Plant, sediment, and water samples from the wetlands were also collected to determine the distribution of fiproles and pyrethroids in the different environmental compartments and to calculate mass balances. Both wetland types demonstrated statistically significant removal of all compounds of interest, with the traditional constructed wetland proving to be a superior treatment option for these hydrophobic contaminants. This result was in agreement with the finding that the compounds under examination were predominantly located in the sediment phase of the constructed wetland, which was not present in the unit process open water wetland.

**279 Why urban and agricultural settings require very different pesticides water quality protection approaches**

*K. Moran, TDC Environmental, LLC*

Pesticides management approaches for water quality protection must address the different upstream pesticide sources, physical environments, and regulatory environments in agricultural settings and urban settings. In agricultural settings, the grower has ultimate control over pesticide applications, often supported by pest control advisors and professional applicators. Growers know the pesticides applied and application timing, can control water use, and have capacity to direct runoff flows from treated fields. In urban settings, thousands of individuals use pesticides in a plethora of ways, sometimes unknowingly (e.g., pesticides impregnated in unlabeled products like paint, roofing, and clothing). Government agencies responsible for urban runoff drainage systems and municipal wastewater (sewage) collection and treatment typically cannot control pesticide use, application location, timing, or discharges in their watersheds and sewersheds. Despite lack of control, under the US Clean Water Act, municipal urban runoff and wastewater agencies (unlike growers) bear legal responsibility for pesticides in urban runoff and wastewater effluent. Pilot projects suggest that treating agricultural runoff containing known pesticides may be technologically feasible, though questions remain about widespread implementation. The outlook for urban pesticides treatment is less promising. With an uncontrolled pesticides mix – and the fact that current use pesticides pass through highly advanced municipal wastewater treatment plants at concentrations exceeding toxicity benchmarks – prospects for treatment as a primary urban pesticide management approach appear poor. Prevention strategies have proven successful in both agricultural and urban settings. Prevention measures occasionally include termination of specific pesticide uses, such as the 1995 California prohibition on sale and use of copper-based root control products in the San Francisco Bay watershed. A more common approach is reducing pesticide runoff load through changes in application rates, locations, and methods, as California Department of Pesticide Regulation and registrants implemented for outdoor structural application methods for pyrethroids (2012) and fipronil (2017). New predictive modeling methods show promise for designing practical prevention-based management practices toward a goal of maintaining pesticide availability for beneficial urban and agricultural purposes without causing water pollution.

## Birds Under Stress: Impacts of Chemical Exposure and Environmental Changes – Part 2

**280 Mercury bioaccumulation in resident and migrant songbirds and effects on body condition**

*J.T. Ackerman, A. Hartman, M. Herzog, US Geological Survey / Western Ecological Research Center*

Methlymercury is a significant risk to environmental health globally. We examined the ecological drivers of methylmercury bioaccumulation in songbirds and its effect on body condition while experimentally removing the potentially confounding and predominant effects of site and habitat. We measured blood and feather mercury concentrations and body condition in nearly 1,200 individuals representing resident or migrant songbirds of 52 species and 5 foraging guilds in the Central Valley of California. Songbird mercury concentrations differed among species, foraging guilds, residency status, dates, and ages, but not sexes. Blood mercury concentrations 1) ranged from 0.003 in house finch to 0.85 µg/g ww in American robin, 2) were 125 times greater in insectivores than granivores and 3.6 times greater in insectivores than omnivores, 3) were 3.3 times greater in summer residents than in migrating songbirds, 4) increased by 25% throughout spring and summer, and 5) were 45% higher in adults than juveniles. Songbird mercury concentrations were negatively correlated with body condition, with blood mercury concentrations decreasing by 44% and 34% over the range of standardized body masses and fat scores, respectively. Our results highlight the importance of foraging and migration ecology in determining methylmercury contamination in birds, and the potential for reduced body condition with methylmercury exposure in songbirds.

**281 Does diet composition or habitat biogeochemistry drive mercury concentration in a threatened wetland bird?**

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Methylmercury (MeHg) is a globally pervasive contaminant with known toxicity to birds. Variation in MeHg concentrations among individuals of the same species may be driven by variation in the biogeochemical pathways involved in MeHg production or by differences in diet composition among individuals. We examined diet composition, trophic structure, and MeHg biomagnification in the food web of a state-threatened, wetland bird, the California black rail (*Laterallus jamaicensis coturniculus*), along with seven measures of sediment biogeochemistry, to determine whether differences in MeHg concentrations among rails from three adjacent wetlands were driven primarily by differences in diet or habitat biogeochemistry. Black rails were dietary generalists with similar diets among wetlands (percent similarity indices > 72%). The trophic structure of the black rail food web was also similar among wetlands, with trophic magnification slopes for MeHg ranging from 0.18 to 0.28. In sediments, the concentration of MeHg and the ratio of MeHg to total Hg (MeHg/HgT) both differed significantly among wetlands ($p = 0.047$ and 0.021, respectively). Further, we identified five sediment measurements that contributed significantly to a discriminant function explaining differences in sediment biogeochemistry among wetlands: loss on ignition, dry sediment MeHg concentration, MeHg/HgT, the ratio of ferrous iron to total iron, and the concentration of total reduced sulfur. Given the similarities in diet composition, trophic structure, and MeHg biomagnification among wetlands, we concluded that variation in habitat biogeochemistry and associated sediment MeHg production was the primary driver of differences in MeHg concentration among rails from different wetlands.

**282 Is mercury influencing the ability of thick-billed murres to adjust to changing ice conditions in the Canadian Arctic?**

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The exposure of wildlife to contaminants can result in endocrine disruption and behavioral changes, and such potential changes may occur for Arctic wildlife in conjunction with rapid climate change. Because the endocrine system plays a critical role in allowing animals to respond to environmental stress (e.g., changing ice patterns), endocrine disruption could limit the plasticity of wildlife to respond to climate change. We are examining these interactions with thick-billed murres (*Uria lomvia*) that feed from ice flows and breed in northern Hudson Bay, Canada. Reductions in the availability of ice mean that the birds must spend more energy, obtain less fish, and this can result in poorer reproductive success and chick growth. We seek to determine whether contaminants compound the impacts of climate change on avian wildlife by limiting their ability to respond to changes in ice availability. In 2016 and 2017, the foraging behaviors and movements of 67 thick-billed murres were tracked using GPS-accelerometers, and concentrations of thyroid hormones, corticosterone, mercury (Hg), brominated flame retardants, and per- and poly-fluoroalkyl substances (PFAS) were measured in the plasma of 47 individuals. Levels of PFASs were low, and unrelated to hormones or behavior. However, mercury levels were associated with circulating triiodothyronine ($T_3$) prior to the birds foraging. In contrast to a “medium-ice” year (2016), in a “low-ice” year (2017), the relationship between $T_3$ and Hg was negative. The $T_3$ concentrations of the birds prior to their foraging trips were associated with their foraging behavior; in contrast to 2016, higher levels of $T_3$ were associated with lower diving rates. We found no associations with plasma corticosterone levels. GPS tracks demonstrated that birds from all sub-colonies foraged to the north of the colony during incubation when ice was present, and then moved to forage to the north-west as chick-rearing progressed and ice was no longer present. These results suggest that birds were foraging near regions of floating ice, which may improve foraging success and reduce diving rates. Based on our 2016-18 data, we tentatively conclude that mercury may be influencing the ability of thick-billed murres to adjust to variation in ice cover, and we will further examine that hypothesis in 2018 with a larger sample size and different environmental conditions.

**283 Does calcium concentration in plasma have a protective effect against metal-related oxidative stress? Experiment in great tit nestlings**

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It has been suggested that calcium (Ca) may provide protection against metal toxicity in the organism. The objective of this study is to explore the effects of Ca levels in the organism and metal exposure on oxidative stress biomarkers in great tit (*Parus major*) nestlings. Great tits were supplemented with Ca (Ca-supplemented group) or not supplemented (Control group) in a metal-polluted and a control area in SW Finland. Feces were collected to measure metal concentrations. We measured an array of oxidative stress biomarkers (GSH, GSH:GSSG ratio, GPx, GST, CAT, SOD, lipid peroxidation and protein carbonylation) in red blood cells and Ca levels in plasma. This study shows that antioxidant levels (tGSH levels, GSH:GSSG ratio, GPx and GST activities) change over the range of metal concentrations depending on the Ca levels in plasma (Fig. 1), suggesting that a balance exists between Ca levels in the organism and the tolerance to metal-related effects. When Ca levels in plasma were higher than 14 mg/dl, increased antioxidants levels were found with increasing metal concentrations in feces. This suggests that birds are able to upregulate their antioxidant capacity to cope with higher metal exposure when Ca in plasma is adequate. On the other hand, when Ca concentrations in plasma were lower than 14 mg/dl, decreased antioxidant levels were observed with increasing metals in feces, suggesting that metals are prone to inhibit the antioxidant system when Ca levels in plasma are low. Thus, Ca concentrations in plasma may have a protective effect against metal-related oxidative stress. Ca scarcity should be considered in future studies evaluating metal exposure and effects in wild birds. *Acknowledgements*: This study was financed by the Academy of Finland (project 265859 to TE), *Societas pro Fauna et Flora Fennica* (to PS-V), University of Turku Graduate School – UTUGS (to PS-V), and *Fundación Séneca* (20031/SF/16 to SE and 19481/PI/14 to AJG-F).

**284 Evaluation of Monotonicity of Concentration Response in Avian Reproduction Studies**

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Avian reproduction tests (OECD 206; OCSPP 850.2300) evaluate effects of dietary exposure to test material for >20 weeks and identify no observed effect concentrations (NOECs). When there is a monotonic trend in response to increasing concentration, the most powerful statistical methods are based on trend tests (*e.g.* Jonkheere-Terpstra (JT) step-down trend test). However, when monotonicity is not evident, investigators often use both trend-based tests and pairwise tests (*e.g.* Dunnett's test (DU)), and make regulatory decisions based on the lowest NOEC. This double testing increases the probability that random differences are declared statistically significant. We evaluated an approach to avoid this double testing. Bootstrap resampling of 36 bobwhite quail reproduction studies and 12 biological measures were used to evaluate the statistical power of tests and the impact of non-monotonicity. Studies were performed in multiple laboratories using a control and 3 exposure groups with 18 pairs of adult quail per group. The resampled data were used to evaluate the performance of DU and JT. When study effects of 20% or less were modeled, random variation alone resulted in ≤1/3 of studies showing greater responses in the low and middle concentrations than both the control and high treatments. When studies with no non-monotonic response pattern were modeled, the power of the JT to detect 20% change is >0.9 for all 12 endpoints except Eggs/Hen/Day (Power ~0.7). Under these circumstances, the JT was slightly more powerful than the DU. If the underlying response pattern is curved so that the responding measure at the highest concentration is reduced by 20% and the mid concentration is reduced by 40%, the power of JT to detect the response in the highest concentration is still >0.9 for all measures except Eggs/Hen/Day Day 14 Hatchling Survivor Weight, and Food Consumption (Power = 0.56, 0.83, and 0.84, respectively); however, the DU is more powerful in detecting effects in all concentrations. We then evaluated the merit of the following rule: 'Use DU test if response measures in low and middle concentrations both exceed those in both control and high treatment groups, otherwise use JT test'. Applying this simple rule resulted in overall power to detect effects equivalent to the DU test alone when the data were non-montonic and to the JT test alone when the data were monotonic, while simultaneously avoiding problems due to double testing.

**285 Interpretation of toxicity data from petroleum dosing studies for integration into avian migration models**

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Assessment of contaminant effects in wild migratory bird populations is generally limited by the time the birds are present in contaminated ecosystems of concern, and end when they fly on to their next destination. However, recent advances in the understanding and modeling of avian migration may provide tools for better prediction of contaminant impacts throughout an extended portion of the annual activity cycle of bird populations. Energetics-based migration models follow bird movements across temporally-relevant, continent-scale maps of food availability while accounting for energy assimilation and expenditure in the modeled birds. Interpreting laboratory and field toxicity findings in terms of their disruption of physiological processes underlying migration could allow prediction of impacts that decrease a bird's ability to maintain energetic conditions that accommodate migration. Toxicological effects that influence migrants' ability to store energy for flight, maintain flight speed, follow migration routes, maintain energetically efficient flight, or carry stored energy during flight could lead to decreased daily travel distances, more frequent refueling stops, and inefficient/erroneous routing to migration destinations. Our current efforts focus on relating previously documented toxic effects of petroleum exposure in birds to endpoints relevant in energetics-based migration models. Previous laboratory and field toxicity studies of birds exposed to oil have shown effects on the hematological, cardiovascular, renal, hepatic, gastrointestinal, endocrine and immune systems. Flight studies have identified impacts on coordination, flight speed and accuracy, energetic expenditures, nutrient assimilation and overall survival. Thermoregulation studies demonstrated loss of body heat with increased nutrient requirements to maintain body temperature. This talk will describe how toxic effects documented in the literature can impact migration-associated physiological and behavioral processes, and thus disrupt migration and threaten survival in exposed birds. Subsequent efforts will focus on incorporation of graded levels of toxic effects into models, as levels of migrant impairment, followed by reassessments of migrant movement, timing, destination acquisition and survival. By understanding the extended ramifications of chemical exposures, we hope to develop more accurate assessments of adverse effects for ecological risk and natural resource damage assessments.

**286 A novel approach to measuring oxidative damage in avian RBCs elucidates an alternative mechanism for cellular damage caused by exposure to crude oil**

*C. Goodchild, Oklahoma State University; S.E. DuRant, University of Arkansas*

Crude oil spills release a variety of contaminants into the environment (e.g., PAHs, toxic metals) and are an ecological hazard to birds and other wildlife. Previous studies in birds have found that exposure to crude oil can cause oxidative damage to red blood cells (RBCs) and hemoglobin (Hb), and may eventually lead to anemia. Although PAHs and toxic metals can damage red blood cells, the mechanism for damage is not always clear. Moreover, common methods for measuring Hb degradation involve using hazardous chemicals. There has been some research in the biomedical field suggesting that oxidation of human-Hb causes degradation of heme subunits and these heme degradation products (HDPs) are fluorescent. In order to better understand the complex relationship between oxidative agents and cellular damage in bird species, we evaluated whether (i) avian RBCs produce fluorescent HDPs, (ii) whether HDPs are linked to RBC membrane integrity, and (iii) whether crude oil exposure increases HDPs. To do so, we collected whole blood from captive zebra finches (*Taeniopygia guttata*) and exposed RBCs in vitro to $H_2O_2$ (model reactive oxygen species). We then measured whether HDPs are correlated with RBC membrane integrity in vivo. Finally, we exposed RBCs in vitro to crude oil and measured HDPs. We found that in vitro exposure to $H_2O_2$ increases HDPs and that HDPs associate with RBC membranes. Additionally, we found that HDPs were linked to impaired membrane integrity in vivo. Furthermore, preliminary data suggest that exposure to crude oil in vitro increases HDPs.

**287 Prioritization of organic contaminants in bald eagles of the upper Midwestern U.S. using the ToxCast High Throughput Screening database**

*S.M. Elliott, US Geological Survey / Upper Midwest Water Science Center; B.T. Route, National Park Service; L.A. DeCicco, US Geological Survey / Office of Water Information; D.D. VanderMeulen, US National Park Service / Great Lakes Inventory and Monitoring Network; S.R. Corsi, US Geological Survey / Upper Midwest Water Science Center; B.R. Blackwell, USEPA / ORD / National Health and Environmental Effects Research Laboratory*

Several organic contaminants, including polychlorinated biphenyls, perfluorinated chemicals (PFCs), polybrominated diphenyl ethers, bisphenol A, and octylphenol have been detected in eaglet plasma collected within and near three national parks in the Midwestern United States. Until recently, there has not been an efficient way to screen these data against toxicity information to provide environmental context or information regarding possible sub-lethal effects. We screened a database of organic contaminant concentrations in eaglet plasma by calculating the ratio of plasma concentrations to concentrations expected to elicit biological responses from the high throughput screening program ToxCast. These ratios, called exposure-activity ratios (EARs), can be used to prioritize contaminants, biological responses, or sites that may warrant further monitoring and research. Bioactivity information was available for 19 of the detected contaminants in eaglet plasma. Most EARs were low ($< 0.1$). However, maximum EAR values were $\geq 1$ for the PFCs, perfluorooctanesulfonic acid (PFOS) and perfluorononanoic acid (PFNA), and bisphenol A in 99, 0.5, and 0.3% of samples, respectively, indicating plasma concentrations were greater than might be expected to elicit some biological response. Genes identified as potentially being affected included those involved in processes such as motor coordination, cardiac functions, behavior, and blood circulation. There was large variability in EAR values across study areas, with larger EARs typically near urban areas. Results from this prioritization assessment can be used to guide future monitoring or research efforts focused on understanding the occurrence and effects of organic contaminants in bald eagles.

## Responsible Chemical Use and Innovative Alternatives for Protecting Infrastructure, Property and Natural Resources

**288 Partnerships for more sustainable chemical use on unpaved roads**

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Chemical treatments on unpaved roads can lengthen road lifespans by stabilizing surfaces and preventing loss of gravel. This protective effect can be particularly beneficial to federal land management agencies that manage extensive networks of unpaved roads with very limited budgets. However, ensuring that chemical treatments are applied in an environmentally responsible way can be difficult. Commercially available products span a huge variety of chemical compositions, including chloride-based, organic petroleum, biobased, synthetic fluid, and electrochemical. Many of these products have little information on ecotoxicity or environmental fate and transport. For some product categories, generating the needed data will require the development of novel testing methods. This presentation describes a collaborative effort among USGS, USFWS, and industry partners to improve the state of environmental knowledge for chemical road treatments. Using a combination of data generated by product vendors for voluntary certification programs (e.g., the former EPA Environmental Technology Verification [ETV] in the United States and Bureau de normalisation du Québec [BNQ] in Canada) and results from a series of laboratory toxicity tests by USGS, project partners were able to identify several products for large-scale field tests at two USFWS refuges. These field tests provided the opportunity to quantify product performance, but also explore novel techniques for evaluating environmental safety under real-world application conditions. In addition, partners developed a two-phase extraction method followed by gas chromatography/mass spectrometry (GC/MS) to detect an environmental signature of a synthetic fluid product that previously had never been tracked in roadside soils. This analytical method has subsequently been adopted by other companies in the private sector. Overall, this project develops a foundation of new environmental data for unpaved road chemical treatments, and provides an example of how cross-sector partnership can help maximize the benefits of chemical treatments while minimizing environmental risk.

**289 Sustainability Implications of Nutrient Removal and Use in Wastewater Treatment Systems**

*M. Sena, A. Hicks, University of Wisconsin – Madison / Civil and Environmental Engineering*

Current use rates of phosphorus have created concern for the sustainability of global phosphate reserves, and research has begun focusing on ways to recover and reuse phosphorus. Wastewater has gained appeal as a viable resource for phosphorus recovery, and the precipitation of magnesium ammonium phosphate ($NH_4MgPO_4$ $6H_2O$),or struvite, has emerged as viable technology. Intentional precipitation of struvite also prevents the unwanted buildup of struvite in other parts of the plant thus reducing and eliminating the expensive maintenance and replacement efforts necessary to remove it. Additionally, struvite precipitation recovers a valuable alternative fertilizer product. However, there are significant energy and chemical requirements necessary for precipitation which raises concern that the overall environmental impacts of this technology are negative.

This research focuses on evaluating the environmental benefits and costs of phosphorus recovery in wastewater treatment by struvite precipitation through a life cycle assessment case study of the struvite precipitation system at a full scale wastewater treatment plant. The considered plant adds magnesium chloride (MgCl?) as a magnesium source and a significant amount of sodium hydroxide (NaOH) for pH control to their struvite precipitation system. Life cycle assessment provides an opportunity to compare the environmental costs associated with the required chemical additions to the benefits from offset production of conventional fertilizers, and conclude weather or not the use of chemicals is justified from an environmental standpoint.

**290 Case Studies on the Use of Chemical Control of Invasive Crayfish**

*A. Allert, USGS / Columbia Environmental Research Center; A.R. Cupp, US Geological Survey / Upper Midwest Environmental Sciences Center; J.A. Stoeckel, Auburn University; K. Knott, Missouri Department of Conservation / Environmental Health*

Integrative pest management programs exploit invasive species life history, target pathways of invasion for invasive species, and use best-available control technologies to combat infestations. Crayfish are critical components of aquatic and terrestrial ecosystem; however, they can be highly invasive. Documented impacts of crayfish invasions include loss of native species of crayfish, amphibians, fish, and birds; alteration of food webs and habitat; and loss of recreation and associated economies. Few chemical control tools are currently available for the management of invasive crayfish. In this work, we investigated the use of cypermethrin, pyrethrin, and carbon dioxide for chemical control of target pathways of new infestations and waterbodies classified as 'non-water bodies' of the state. Laboratory and field investigations tested control of adult Red Swamp Crawfish (*Procambarus clarkii*), White River Crawfish (*Procambarus acutus*), Rusty Crayfish (*Faxonius rusticus*), and Virile Crayfish *(Faxonius virilis*). Research included collaborative interactions with state managers and regulatory agencies to ensure the protection of non-target species and ecosystems, and the registration and permitting for the application of the test chemicals. The results will inform resources managers on best practices for the application of chemicals singly or in addition to mechanical and biological control methods for the control of invasive crayfish.

**291 Promising practices for making informed chemical selections using alternatives assessment**

*A. Nestler, C. Trebilcock, L. Heine, Northwest Green Chemistry*

Alternatives Assessment (AA) provides a framework for informed decision making for intentional chemical applications with transparent analysis of benefits & trade-offs. AA is defined by the Interstate Chemicals Clearinghouse (IC2) as a process for identifying and comparing potential chemical and non-chemical alternatives that can be used as substitutes to replace chemicals or technologies of high concern. AA is an excellent method for identifying the safest solution, be it intentional chemical application or an alternative. In support of WA legislation that phased out the use of copper in recreational antifouling boat paints, the independent nonprofit Northwest Green Chemistry (NGC) recently completed an AA of antifouling technologies, the first AA published following the IC2 AA Guide. NGC is currently engaged in AAs looking at bridge coatings, fluorinated coatings on single-use foodware, and phthalates. This presentation is a retrospective of our AA work, and we will share promising practices, potential pitfalls, and opportunities to influence policy with AA. AA can influence policy and implementation of disruptive solutions. First, the AA recognized the lack of any alternatives for boats with wooden hulls, which may receive an exception. Second, the AA revealed a potential regrettable substitution: Elevated zinc oxide concentrations in copper-free alternatives, which NGC is currently assessing. Third, work with stakeholders provided opportunities for them to meet with representatives of disruptive solutions, such as the Drive-in Boatwash, and explore adoption. From the perspective of promising practices, stakeholder engagement was invaluable. Stakeholders helped identify available alternatives, refine criteria and metrics for product evaluation, identify preferred communication methods, and explain adoption challenges they would face in the transition. Furthermore, involving stakeholders in designing criteria & metrics increases the likelihood that they will accept and support the results of the AA. From the perspective of potential pitfalls, data gaps challenged the AA team in the performance module. The recreational market is not subject to standard test requirements such as those specified for government purchasing, resulting in a paucity of high-quality data. Our report revealed promising practices & potential pitfalls of AA. The AA framework, as a transparent decision-making tool, can be used to clearly communicate with policymakers and the public.

**292 Sustainable Microbial Control Technologies: Preservation of Environmental Resource Quality During Oil and Gas Extraction**

*C. Lehman, Dow Chemical Company / Industrial Biosciences*

As conventional practices for hydrocarbon extraction become less productive, reliance on new sources, including oil sands, tight gas shales, coal bed gas and shale oil is gaining prevalence. Novel extraction techniques, like hydraulic fracturing, are often considered sources of environmental damage and contamination. However, many of these negative effects can be minimized through the strategic targeted use of biocidal products, which allow for optimization of production rates, a reduction in the volume of functional fluids needed, and ultimately a focus on maximizing water preservation and minimizing environmental impact both onshore and offshore. Techniques such as the utilization of biodegradable biocides and diagnostic DNA profiling of target microbial populations can further reduce exposure of terrestrial and aquatic organisms to biocidal actives. Biocides are a highly regulated, well-studied group of chemicals that have been heavily scrutinized with respect to hazard, exposure, and risk mitigation. When this understanding is coupled with risk-based characterization tools, which are scientific criteria that consider both hazard and exposure, biocides can sustainably and effectively control microbial contamination during oil and gas extraction activities.

**293 Bioassay testing applications for products and stewardship**

*P. Arth, Enthalpy Analytical / Nautilus Environmental / Aquatic Toxicology*

Bioassay testing has been utilized to assess the potential toxicity of finished products for many years. Recently, with increasing implementation of the Globally Harmonized System (GHS) of Classification and Labeling of Chemicals, bioassay testing has become a more prominent part of safety data sheet (SDS) preparation. There are additional applications of bioassay testing for products and commercially manufactured goods that can be useful assessment and decision-making tools beyond the commonly recognized framework. Bioassay methods can be a valuable tool for evaluating the potential toxicity of a finished product or individual components, newly developed chemicals, and existing products adapting to changing standards and scrutiny. One such application is the use of bioassays in evaluating ecotoxicological impacts of formulaic modifications. In cases of modifications including the addition of new ingredients or changes to ratios of existing ingredients, bioassays can provide an integrated assessment tool, providing a clearer picture than reliance on literature-based effect levels. Bioassay testing methods can also serve as tools to evaluate the merit and veracity of environmentally-conscious marketing content. Scientists with a deep understanding of these tools and their appropriate use and limitations can support goal definition, testing design, and generation of legally defensible data, which can be incorporated into product development strategies and used as a competitive marketing advantage or a basis for public communication. While some comparative values exist, evaluating products concurrently under identical conditions provides a measure of certainty and data fidelity. Bioassays are also an integral part of the registration process and ensuring this expertise is represented on the registration team helps to ensure that the required data are generated for what is often a complex, lengthy, and resource-intensive process. Given the lack of specific method accreditation, latitude permitted by many test methods designed to evaluate products and chemicals, and overlapping jurisdictional authority and guidance, understanding the potential experimental design challenges associated with a given test article is critical to ensuring an accurate outcome and acceptable data submissions. This is a developing arena for thoughtful application of these tools and an opportunity to prevent environmental impacts and create commercial value for responsible stewardship.

### 294 Towards Sustainable Alternatives for Long-Chain PFAS in Firefighting Foam

*T. Bruton, A. Blum, Green Science Policy Institute*

The use of aqueous film-forming foam (AFFF) in firefighting activities has resulted in environmental releases of poly- and perfluoroalkyl substances (PFAS), adversely impacting the drinking water supplies of communities around the world. Regulatory actions to date have focused on long-chain perfluoroalkyl acids, a subgroup of PFAS including perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), due to their widespread detection in a variety of matrices including human serum, as well as their demonstrated hazard properties. For example, USEPA negotiated the phase-out of PFOS and PFOA with major manufacturers and issued drinking water guidance for both chemicals. As long-chain PFAS-based firefighting foams have been phased out in the U.S., they have been replaced by AFFF containing short-chain PFAS, despite the similar chemical properties of the two groups and the availability of PFAS-free alternatives. The adoption of foams containing short-chain PFAS rather than fluorine-free foams was driven by multiple factors, some of which are scientific, others of which reflect the interests or values of various stakeholders. This talk will compare and contrast the chemical and toxicological properties of long-chain and short-chain fluorinated surfactants used in firefighting foams, as well as the newer fluorine-free alternatives. The presentation will also explore the environmental and firefighting performance criteria that resulted in the adoption of foams containing short-chain PFAS in the U.S. The evolution of firefighting foam use in the United States will be compared with what has happened internationally. Understanding the full context in which chemicals policy decisions such as this one are made can help scientists and policymakers reduce the potential for regrettable substitutions and find sustainable chemical alternatives.

### 295 Toxicity of wildland fire-fighting chemicals following short, pulsed exposures

*H.J. Puglis, C. Mackey, E.L. Scott, S. Tidwell, R. Calfee, US Geological Survey / Columbia Environmental Research Center*

During the application of fire-fighting chemicals, misapplications can occur such that chemicals are inadvertently applied to the surface of water. The environmental safety of fire-fighting chemicals is most often based on laboratory studies that determine the concentration of chemical lethal to 50% of test organisms over a continuous 96 hour exposure. In contrast, real-world misapplication may result in relatively brief pulsed exposure to an initially high concentration that is progressively reduced as the retardant is diluted and washed downstream. Additionally, pulsed exposure conditions can occur following precipitation events when fire-fighting chemicals applied to the landscape and riparian areas can runoff into waterways. The purpose of the study was to understand the potential toxicity of fire retardants to rainbow trout (*Oncorhynchus mykiss*) after short, episodic exposures. To simulate these short term exposures under different flow scenarios, 30-60 day post swim-up trout were exposed to 1 of 3 different fire retardants, Phos-Chek® MVP-Fx, Phos-Chek® 259-Fx, or Phos-Chek® LC-95A-R, for 15 minutes or 1 hour at two exposure concentrations and then placed in control water to evaluate recovery. The concentrations tested varied for each chemical but include the range of application rates used during fire-fighting activities. After 48 hours in the control water, half the replicates were exposed to the same chemical for the same exposure concentration and duration, and then placed back in the control water. Average mortality was below 20% in all treatments. Survival of the trout varied by number of exposures, chemical tested, and exposure duration, with generally lower survival in treatments experiencing multiple exposures for longer durations. These data can be used by resource managers in selecting products for application to manage any potential risks to aquatic organisms associated with misapplication.

## Incorporating Effect-Based Molecular Assays in Environmental Monitoring and Risk Assessment

### 296 Bioanalytical evaluation of advanced wastewater treatment technologies: A systematic review of existing toxicity data

*J. Völker, NTNU University / Biology; M. Wagner, Norwegian University of Science and Technology / Department of Biology*

Due to the ineffective removal of a broad spectrum of compounds, the discharge of conventionally treated wastewater can negatively impact surface waters and may also affect the quality of raw water for human consumption. Accordingly, several countries either consider or have already started to upgrade their wastewater treatment plants (WWTPs). Full-scale trials conducted at WWTPs demonstrate that technical solutions such as ozonation or activated carbon treatment reduce the load of various selected micropollutants. Nevertheless, an evaluation of wastewater treatment technologies based on few target compounds does not address the large number of micropollutants, their mostly unknown transformation products as well as the combined toxicity of the complex mixtures. To address these limitations, effect-based measurements are increasingly integrated into the evaluation of advanced WWTPs. Bioassays provide a more comprehensive picture of their performance in removing toxicity rather than single chemicals. However, while several reviews of target micropollutant removal by advanced wastewater treatment technologies exist, a systematic review of the removal of toxicity during wastewater treatment is lacking. We, therefore, analyzed the outcomes of existing literature data. Based on our selection criteria, we identified 28 relevant studies containing data of 52 different in vitro bioassays targeting a broad spectrum of endpoints including several endocrine endpoints, induction of xenobiotic metabolism, insecticide and herbicide marker, adaptive stress response, unspecific or baseline toxicity as well as genotoxicity and mutagenicity. Based on this extensive data set, we will give an overview of applied methods, summarize consistent and contradictory results, and highlight research gaps. Moreover, to evaluate the performance of the applied technologies, we will discuss the data set in the context of recently proposed effect-based trigger values.

### 297 Using High-Throughput Approaches to Assess Effectiveness of Wastewater and Stormwater Treatment

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We evaluated whether advanced treatment of wastewater (disinfection) and stormwater effluents (iron enhanced filtration) improved removal of contaminants of emerging concern and associated toxicities. Workflow for two studies was similar. Composite water samples were collected from pre-, and post-treated effluents. Their chemical composition and the effects on a variety of molecular targets (25 human nuclear receptors; NRs) and 46 transcription factors/pathways; FACTORIAL platform, Attagene, Inc) were evaluated. Chemistry data was integrated with the publically available toxicity data (ToxCast) to predict whether treatment-related decreases (and increases) in chemical concentrations would “make any difference” to biological receptors. Outcomes of these “in silico” predictions and the empirical in vitro data were compared for consistency.

Control water, and pre- and post- advanced treatment effluents were also used to conduct short-term exposures with fish (wastewater) or *Daphnia* (stormwater); transcriptomic and metabolomics analyses of these samples are partially completed. In silico predictions identified nuclear receptors (well-characterized targets such as estrogen receptor in particular) as the most sensitive and likely to be affected by the exposure; empirical in vitro data supported this prediction. However, in vitro data pointed to additional chemical drivers of toxicity; many of the molecular targets covered by ToxCast were not indicated by predictive toxicology analyses, but were activated in vitro (e.g., peroxisome proliferator activating receptor (PPRE) and glucocorticoid receptor (GR)). In vitro and in vivo toxicity assessments also indicated that treatment-related reductions of parent chemicals are not always paralleled by toxicity reductions. These findings emphasize the importance of characterizing transformation products associated with the wastewater disinfection and stormwater treatment, and highlight the advantages of combining analytical and high-throughput bioeffects approaches to evaluate treatment effectiveness. We are currently integrating analytical chemistry and empirical toxicity data using association rule learning to advance understanding of chemical drivers of wastewater and stormwater toxicity.

**298 Quantitative toxicogenomics methods to predict adverse outcomes in fathead minnow**

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Methods of measuring whole-genome gene expression changes, such as microarrays and RNA-sequencing, have great potential for applications in screening and prioritization of environmental contaminants by regulatory agencies. Transcriptomics data can be analyzed to determine whether exposure to a contaminant induces changes in gene expression that have been associated with the development of adverse outcomes. However, the integration of transcriptomics data into regulatory decision making has been hindered by the lack of robust quantitative methods that consistently link well-defined changes in gene expression to apical outcomes. Previous efforts to address this issue have attempted to summarize groups of individual genes in pathways or modules, however changes in the expression of these gene sets have rarely been explicitly linked to apical outcomes. Here, we hypothesize that data-driven gene selection methods will produce toxicity modules with higher predictive power for a well-defined set of apical outcomes than toxicity modules limited to include genes from established molecular pathways. The objective of this study was to define three different sets of 25 toxicity modules for fathead minnow; the first derived from KEGG pathways, the second derived from clusters detected in a co-expression network, and the third derived from a hybrid KEGG/co-expression network approach. The KEGG pathways were chosen based on discussion with toxicology experts and end-users and include 230 individual KEGG pathways from 5 of the 7 KEGG pathway databases. These KEGG pathways form the basis of the co-expression analysis, which is underway. The underlaying dataset being used to build the co-expression network is composed of Agilent-019597 microarray profiles ($n = 304$) from seven previously published studies that measured hepatic gene expression after exposing adult fathead minnow to estradiol, RDX, diethylstilbestrol, PFCs, phenanthrene, and effluent from eight waste water treatment plants. The reproducibility and ability of the three sets of toxicity modules to predict apical outcomes will be evaluated with our own measured RNA-sequencing and apical outcome data from exposure of adult and early-life stage fathead minnow to two model endocrine disrupting chemicals (ethinylestradiol and chlorpyrifos). This study is part of the EcoToxChip project (www.ecotoxchip.ca).

**299 Comparing nanosilver-induced toxicogenomic responses of rainbow trout and white sturgeon using whole transcriptome shotgun sequencing**

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Silver nanoparticles (AgNP) is extensively used due to their wide spectrum of antimicrobial properties, but their continuous discharge to surface waters pose risks, particularly towards non-target organisms. In particular, little is known about the toxicological significance of AgNP to fishes native to North America. Typical read-across tests may help informing known toxic responses but may fail to detect other relevant effects, hence, there is a need to generate unbiased information by probing entire biological systems without a priori knowledge of the mechanisms of toxicity. Recent advances in ‘omics technologies have the potential to address this need as they offer high-throughput and cost-effective approaches as they allow for the unbiased examination of patterns of mechanisms of toxicity for use in predictive environmental risk assessment (ERA). Therefore, the objective of this study was to evaluate cross-species responses in native fish species, rainbow trout, *Oncorhynchus mykiss* (RBT) and white sturgeon, *Acipenser transmontanus* (WS), using whole transcriptome-based toxicity pathway analysis. Juvenile RBT and WS were exposed to 10 nM of PVP-coated AgNP in 96h static-renewal system, and sequence-by-synthesis whole transcriptome analysis were used to determine differential gene expression patterns in fish livers. A 0.05 cut-off false discovery rate (FDR) identified differentially expressed contigs between AgNP and control groups. A total of 314 and 415 contigs with gene names were significantly altered in RBT and WS, respectively. Gene set enrichment and pathway analysis using ontologies based on KEGG and GO Consortium showed 23 and 16 pathways perturbed in RBT and WS, respectively. However, only 4 pathways were common between the two species. These pathways include platelet activation, steroid biosynthesis, and integral membrane component and protein processing in ER. On the other hand, using p-Value as less strict parameter in gene selection expanded the list of dysregulated genes and perturbed pathways. Over half of the pathways perturbed in WS overlapped with RBT and were mainly involved in metabolism, biosynthesis, and transport. The results of this study will be compared to apical outcomes assessed in a parallel chronic study, and which will allow the assembly of toxicity pathways across multiple levels of organization with the end goal of identifying molecular markers that are indicative of adverse effects.

**300 Linking transcriptomic responses to apical outcomes of chronic chlorpyrifos exposure in Xenopus laevis**

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Chlorpyrifos (CPF) is a widely use organophosphate insecticide with extensive occurrence in aquatic ecosystems worldwide. Exposure to CPF is associated with adverse effects in fish including developmental delays and abnormalities, oxidative stress, neurotoxicity, and immunotoxicity. There is much less known regarding the chronic, low-level exposure effects of CPF in amphibians. The objectives of this study were to (1) evaluate apical outcomes of chronic exposure to CPF on the model amphibian (*Xenopus laevis*) and (2) identify and link key molecular response patterns that may be altered during exposure to CPF to these apical outcomes of regulatory relevance. Tadpoles were exposed to CPF (0.5, 2, 8 ug/L, nominal) from early life stage (immediately post-hatch) through to metamorphosis. Individuals were sampled after 96 h and assessed for whole body transcriptome (RNASeq). A subset of tadpoles was then transferred to a flow-through diluter system for exposure to metamorphosis (~ 55 d) and assessed for developmental stage, morphometrics, and organ histopathology. There was no effect of chronic CPF exposure on morphometric endpoints including length, weight, relative liver weight or incidence of malformations. Individuals exposed to 2 ug/L CPF were at a more advanced developmental stage compared to the solvent control. Apical effects will be assessed through histopathology of key organs as well as measures of oxidative stress, immune and apoptotic markers. We anticipate that this work will provide new and valuable amphibian CPF toxicity data to compare to well-studied fish species. We anticipate that this work will identify early life stage critical toxicity pathways that could be used to predict apical outcomes of ecological and regulatory relevance in amphibians, providing an alternative approach in chemical screening. This study is part of the EcoToxChip project (@ecotoxchip).

**301 Coupling animal behavior and gene expression to develop new tools for pharmaceutical environmental risk assessment**

*I. Huang, N. Dheilly, Stony Brook University / School of Marine and Atmospheric Sciences; H. Sirotkin, Stony Brook University / Neurobiology and Behavior; A. McElroy, Stony Brook University / School of Marine and Atmospheric Sciences*

Pharmaceuticals and personal care products (PPCPs) are a diverse group of emerging contaminants that are increasingly detected in surface waters worldwide. While environmental concentrations are typically low, recent research has shown that aquatic organisms residing in contaminated waters bioaccumulate many of these contaminants in various tissues. Despite these measurable body burdens, it is unclear what physiological or ecological impacts PPCPs might have. Long term, sub-lethal effects are difficult to study as many molecular techniques may not be sensitive enough to capture signals of chronic toxicity. Both gene profiling and animal behavior have been proposed to act as sensitive indicators of sublethal toxicity and are both promising methods to approach low level emerging contaminants such as PPCPs. The research we present here couples behavioral analysis with gene expression profiling to develop biomarkers of sublethal antidepressant pharmaceutical exposure and effect. We exposed zebrafish (*Danio rerio*) embryos and larvae to two popular selective serotonin reuptake inhibitor (SSRI) antidepressants commonly found in the environment, fluoxetine (Prozac) and paroxetine (Paxil), and found significant changes in spontaneous swimming behavior and visual motor response at six days post fertilization. RNA-sequencing of individual larval zebrafish brains identified over 1500 genes that were significantly differentially expressed in response to SSRI exposure at concentrations demonstrated to significantly alter swimming behavior. By mining the RNA-seq data, we identified 8 candidate genes that can potentially be used as biomarkers for SSRI exposure and behavioral alteration (fkbp5, nfil3-5, pfkfb4b, ucp2, foxk1, cry1bb, klf9, and stk35). Currently, qPCR assays are underway to confirm that the expression patterns of these 8 biomarker genes are reproducible between different gene expression profiling methods and to test their dose-response and time varying behavior. Further performance tests will include exposing larval zebrafish to wastewater effluent extracts (which contain both SSRIs among other contaminants) to test the efficacy of these biomarkers in realistically complex matrices. Our hope is to integrate molecular biomarkers with other indicators of sublethal toxic effect, like animal behavior, to eventually create predictive models of toxic effect, which will help improve environmental risk assessment and management.

**302 Critical considerations in detecting environmental DNA (eDNA) for enhanced population effects assessment**

*C.C. Helbing, University of Victoria / Biochemistry & Microbiology; J.M. Round, M.J. Allison, L.C. Bergman, University of Victoria*

In toxicological paradigms such as the Adverse Outcome Pathway (AOP), the ultimate desire is to link molecular and organismal indicators to population effects. Evaluation of populations has largely relied upon traditional time-constrained searches that are particularly challenging for species that are cryptic and/or at low densities. The use of environmental DNA (eDNA) detection as a means to assess population distribution and dynamics is becoming increasingly popular as it is more sensitive and cost-and time-effective than traditional methods. eDNA is any trace fragment of exogenous DNA that is released by an organism into the environment. By obtaining a water sample, information about the presence or absence of a species can be determined by measuring the DNA contained within. Targeted species eDNA detection relies upon the use of primers and probes within the context of quantitative real time polymerase chain reaction (qPCR). While considerable thought has been placed on eDNA field collection methods, comparatively little scrutiny has been directed to the qPCR-based tests for which eDNA detection poses unique challenges. Critical considerations include proper primer/probe design and validation for specificity and sensitivity, assessment of DNA sample integrity, and the incorporation of appropriate field- and lab-based controls. Based upon experience from over 40 projects and thousands of samples, these factors will be discussed with solutions for enhancing confidence in eDNA testing methods used in environmental assessment and monitoring.

**303 Mechanistic Tools in Contaminant Monitoring and Risk Assessment--Addressing the Disconnect**

*G.T. Ankley, USEPA / National Health and Environmental Effects Research Laboratory*

In 1989 the Society of Environmental Toxicology and Chemistry sponsored a Pellston workshop focused on the use of biochemical, physiological, and histological measurements for monitoring the occurrence and effects of contaminants in the environment. The outcome of that workshop stressed the many advantages of these types of mechanistic endpoints (e.g., rapidity/ease of measurement, diagnosis of specific stressors, provision of an early warning system, etc.), and highlighted impediments to their practical adoption and widespread use. Over the past three decades, the scientific community has made huge strides in the ability to generate mechanistic data through advances in areas such as transcriptomics, proteomics, metabolomics, and high throughput (HTP) in vitro testing, but there has been much less progress in terms of routinely using this type of knowledge. So, where have we succeeded and where have we fallen short? Progress has been made in areas such as technical information transfer and the development of approaches for translating mechanistic data into responses meaningful to risk assessments. Other challenges have proven more complex and difficult to address. For example, to employ novel mechanistic tools/concepts, case studies are needed to demonstrate that results/decisions using newer approaches are of a quality comparable to (or better) than current techniques. However, this type of work can be very resource-intensive and the studies difficult to design/interpret with a high degree of confidence. Contributing to the difficulty in demonstrating "success" in case studies is balancing expectations of users with what can be realistically achieved. This presentation will address where we have made progress relative to use of mechanistic data in chemical risk assessment, and identify areas where further work is needed. *The contents of this abstract neither constitute nor necessarily reflect USEPA policy.*

## Interpreting Biological Effects of Metals and Their Mixtures in the Aquatic and Terrestrial Environments

**304 Field biomonitoring and stream mesocosms to evaluate aquatic insect life stage responses to acid mine drainage**

*C. Kotalik, W. Clements, Colorado State University / Fish, Wildlife and Conservation Biology*

North Clear Creek (NCC), Colorado, USA, is severely degraded by dissolved metals and metal oxides, but recent installation of an acid mine drainage (AMD) treatment facility has dramatically lowered metals concentrations. Of particular interest is the trajectory of benthic recovery in NCC following improved water quality. We tested the hypothesis that different aquatic insect life stages, larvae versus winged-adults, would differ in their recolonization propensity. Benthic and emergence sampling were conducted along the NCC metals gradient, and stream mesocosms were used to compare differences in life stage responses to metals under experimentally controlled conditions. Field results showed higher abundances of adults compared to larvae occurring at impaired sites, but higher densities of larvae compared to adults were observed at reference sites. These differences may be the result of source populations from less degraded sites above and below the impaired reaches contributing adults, while conditions at impaired sites still cannot support high larvae densities due to metals inputs from non-point sources and sediments, and poor substrate quality due to historic metal-oxide deposition. Mesocosm results from the dissolved metals (Cu + Zn) experiments revealed greater reductions in larvae abundances at lower metals concentrations compared to emerging adults. However, larvae and adult emergence were similarly associated with the Fe(III) concentration gradient, and adult blackfly emergence was actually more sensitive compared to larval mortality. Differences in response between the metals stressors was likely related to their routes of toxicity, with physiological accumulation of free metal ions associated with dissolved metals, compared to the physical smothering associated with the metal-oxide treatments. Together, these field and mesocosm results suggest that aquatic insects have life-stage-dependent responses to metals, and traditional bioassessments of larvae may inaccurately predict the responses of adults. Aquatic insect adults are critical to benthic recolonization, overall population persistence, and are an important food subsidy to reciprocal terrestrial ecosystems. Field and laboratory approaches should critically evaluate both larvae and adult life stages in order to better understand differences in their responses to AMD exposure and their contribution to benthic recovery.

**305 Using toxicity and amendment studies to inform risk management of containment pond water containing metal mixtures associated with uranium mining**

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The Grand Canyon watershed in northern Arizona, USA contains relatively high-grade uranium deposits associated with breccia pipe formations. The USGS and its partners are currently studying several breccia pipe sites in various life stages (e.g., pre-mining, active-mining, post-mining/pre-reclamation) in order to provide information on the biological and ecological effects supporting risk-based evaluation of breccia pipe uranium mining. Water produced from the mine shaft and runoff from the overburden and ore pile is held in a containment pond. In this work, the *Ceriodaphnia dubia* chronic toxicity bioassay was conducted using waters collected from a pre-mining site containment pond. At the time of water collection, mine shaft drilling was nearing, but had not yet reached the U ore body. The purpose of this study was to determine pond water toxicity to *C. dubia*, identify the metals responsible for any observed toxicity, and to identify potential treatment amendments that could be used to reduce the bioavailability of metals at the site as part of site management. Chemical and bioassay results identified arsenic, cobalt, copper, nickel and uranium as potential risk drivers at the site. Further toxicity studies were conducted for the individual metals (As, Co, Cu, Ni, U) using laboratory waters that mimic pond water quality characteristics. Uranium toxicity in the laboratory water was not observed at the relatively high levels observed in the containment ponds. Geochemical modeling using PHREEQ-C determined that uranium in the waters was likely not bioavailable due to the relatively high levels of calcium, magnesium, and high water hardness but rather a result of elevated As, Co, Cu, and Ni. Batch and isotherm studies using biochar, clinoptilolite zeolite, and glauconite clay amendments were conducted to determine optimal treatment to reduce toxicity by reducing concentrations of free/dissolved metals in the water. Reconstituted metal mixtures in laboratory waters eluted from amendment-packed columns were used to perform the *C. dubia* toxicity bioassay. Results from the toxicity, geochemical modeling, and amendment studies are intended to guide management of metal mixtures in containment pond waters at breccia pipe U mines.

**306 Assessing Bioavailability of Chromium and Nickel in Watercourses, Wetlands, and Floodplains Using Multiple Lines of Evidence**

*S.M. Jones, D.W. Smith, GHD*

Concentrations of chromium and nickel well above default clean-up levels were detected in sediment of a small stream, and wetland/floodplain soils at a Brownfields site in central New York. Sampling of the water column identified very low concentrations of both metals, suggesting that neither metal was likely to be bioavailable or pose risk to ecological receptors despite very high concentrations in bulk sediment and soil. Evaluation of risk to ecological receptors considered five lines of evidence, all of which demonstrated low bioavailability. Porewater in the stream sediments was collected using passive sampling devices (peepers) deployed over a two week period. Concentrations of both metals in porewater were significantly below toxic levels. Risk to the plant communities of the wetlands and floodplain forest was evaluated using two lines of evidence: toxicity tests for germination and emergence of lettuce seeds and vegetative index of biotic integrity (VIBI). The toxicity tests identified a positive correlation between concentrations of chromium and nickel and both test endpoints (i.e., performance increased with increasing concentrations). The VIBI documented healthy plant communities in the wetlands and floodplain. Analysis of earthworm tissue provided a fourth line of evidence to document low bioavailability. Lastly, leaching analyses showed very low leachability of either metal from soils. The results were used to develop site-specific clean-up levels for chromium and nickel for protection of ecological receptors significantly higher than the default levels.

**307 Predicting recovery of aquatic ecosystems after mine reclamation: Mesocosms and in-stream experiments on the North Fork of Clear Creek, Colorado**

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The construction of a mine effluent treatment plant in Blackhawk, Colorado, has potential to markedly improve water quality in the North Fork of Clear Creek (NFCC). Upstream of Blackhawk, biomonitoring has shown fish, algae and macroinvertebrate populations to be healthy. However, immediately downstream of the first mine adit aquatic life and function was largely limited by metal pollution. The restoration efforts in Blackhawk present a novel opportunity to develop tools to predict the recovery of aquatic life to the North Fork of Clear Creek and other ecosystems stressed by mixtures of toxicants. Several in-stream experiments were conducted to predict response of fish, aquatic insects and algae. Fish cages across a gradient of pollution were used to predict what species and age classes of trout could survive in NFCC after restoration. Pulse Amplitude Modulated Fluorimetry was used to assess colonization of substrate by algal communities. Metal concentrations were modeled using conductivity and depth data loggers to better capture natural fluctuations in metal concentrations that are often missed by daily or weekly sampling. Fish survival, fish drift behavior and pulse amplitude modulated fluorimetry of periphyton were used across a gradient of metal mixtures to determine at what level of restoration these populations might survive. These tools can be used to predict what species are best for reintroduction to restoration sites and study effects of metal mixtures and fluctuations in an environmentally relevant setting. Results of field studies and mesocosm experiments designed to predict recovery of aquatic life to NFCC often differed from results of laboratory trials. Mesocosm studies using mixtures of toxicants that match NFCC showed response of some aquatic insect and fish taxa below levels predicted by laboratory trials. We found functional responses such as algal respiration and colonization to be more sensitive endpoints than the loss of sensitive species. Even with the reduction in aqueous metals, the indirect effects of iron oxides might preclude colonization of some aquatic life.

**308 Can we model both the bioaccumulation and toxicity of metal mixtures using the same set of parameters in the biotic ligand model?**

*Q. Tan, S. Lu, Xiamen University / College of the Environment Ecology*

The biotic ligand model (BLM) is a useful tool for predicting the bioavailability of metals. It can be used for simulating the effects of water chemistry on either metal bioaccumulation or metal toxicity, and usually separately in different studies. Therefore, even for the same combination of metal and organism, two different sets of BLM parameters would be generated, sharing the same symbols (e.g., KCuBL, KCaBL) but having different values. It has not been tested whether it is possible to obtain universal parameter values for both scenarios. Moreover, there are ongoing efforts to extend BLM for simulating the bioaccumulation and toxicity of metal mixtures, which raise the same question again. In this study, we tested the feasibility of using a single set of BLM parameters to explain both the bioaccumulation and toxicity of mixtures of cadmium (Cd) and zinc (Zn) in an estuarine clam Potamocorbula laevis. The assumptions of our mixture BLM include: (1) Cd2+ and Zn2+ are internalized through the same biotic ligands (i.e., transport sites); (2) Cd2+ and Zn2+ compete with each other during internalization; (3) Internalized Cd and Zn exert toxicity independently. Metal bioaccumulation were quantified using a stable isotope tracer technique; toxicity tests of the Cd-Zn mixtures were conducted in parallel. We found significant interactions between Cd and Zn in both the bioaccumulation experiments and the toxicity tests, consistent with the prediction of BLM. Analyzing the data under the framework of a toxicokinetic-toxicodynamic model using BLM as the toxicokinetic module, we obtained a set of parameter values that could well explain all of the bioaccumulation and toxicity data. The model can be further used to separately quantify the contribution of Cd and Zn to the overall toxicity of the mixture. Through this work, we demonstrated that a unified BLM could be developed to model the interaction between metals in their mixtures.

**309 Predicting nickel and copper toxicity to plants: A meta-analysis**

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Copper and nickel are both essential trace elements that are used as co-factors for plant enzymes. However, they can result in toxicity above threshold levels, and this effect varies with the nature of the element as well as the plant species. The present meta-study reanalyzes 16 published datasets to investigate if (1) bulk free ion activity ($M2+_b$) or plasma membrane free ion activity ($M2+_0$) best explains observed toxicity to Ni and Cu in variety of plant species and water chemistries; and (2) does a model based on plant metal binding specificity (WHAM-FTOX) or one based on physiological effect at the plant surface (ETM) better explain observed toxicity to Ni and Cu in a variety of plant species. In addition, for the studies that simultaneously measured metal accumulation in the plants, we test whether $M2+_b$, $M2+_0$ or M-FTox predict M-BL, with the assumption that the accumulated metal is equivalent to metal bound to the biotic ligand (M-BL). The present study focused mainly on toxicity datasets from hydroponic plant cultures in order to avoid confounding factors such as inter-correlations among co-variate parameters. The overall goal is to provide the scientific support for existing modeling tools as a cost-effective alternative to mass toxicity testing for the purposes of risk assessment of metal-impacted regions.

**310 Determining Water Quality Threats to *Acropora palmata* Reproduction at Salt River Bay National Historic Park and Ecological Preserve in St. Croix, USVI**

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*Acropora palmata* (elkhorn coral) was historically one of the most abundant reef building corals in the Caribbean, but they have declined more than 80% in the last 30 years. In addition to rising sea surface temperatures, ocean acidification, white band disease outbreaks and storms, *A. palmata* and shallow water corals are particularly threatened by anthropogenic pollution and degraded water quality. The surviving *A. palmata* in Salt River Bay National Historic Park and Ecological Preserve (SARI) in St. Croix, USVI have shown reproductive impairment. In contrast, *A. palmata* from Buck Island Reef National Monument (BUIS), northeast of the St. Croix mainland, have a greater reproductive output at most sites. One hypothesis is that poor reproductive condition is attributed to poor water quality in the vicinity of SARI. Sediment and coral biopsies from SARI and BUIS were analyzed for trace elements using ICP-MS to measure metals and elemental proxies of water chemistry parameters. When comparing the SARI population to the BUIS population, boron (B), barium (Ba), and phosphorus (P) were greater in the BUIS *A. palmata* coral skeletons. These elements are proxies of pH, freshwater runoff, and nutrient input, respectively. This analysis infers that nutrient input and freshwater input from the surrounding watershed are not correlated to poor reproductive output in SARI *A. palmata.* In the SARI population lead (Pb), zinc (Zn), and iron (Fe) were significantly greater in *A. palmata* coral skeletons, which indicates greater exposure to anthropogenic pollution and sedimentation in SARI, with concentrations comparable to other studies. Since higher concentrations of Fe were found in *A. palmata* skeletons at SARI, further research should compare sedimentation rates in SARI and BUIS and examine the effects of chronic sedimentation on *A. palmata* reproductive output.

**311 Engineered nanoparticles increase excretion rates of nitrogen and phosphorus by freshwater snails in wetland mesocosms**

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Wetlands are exposed to engineered nanoparticles through discharge from wastewater and agricultural runoff. We conducted an experiment to examine the combined effects of chronic dosing of nanoparticles and nutrients on wetland food webs. Three levels of nanoparticles (control, gold, copper) were crossed by two levels of nutrients (ambient vs. chronic nutrient dosing) in 18 outdoor wetland mesocosms. We measured the rate of excretion of nitrogen (N) and phosphorus (P) in freshwater snails after six months exposure to chronic nanoparticle and nutrient dosing. Ten *Physella acuta* snails were collected from each mesocosm. Individual snails were placed into tubes containing filtered, control mesocosm water. Snails were incubated for 95 ± 0.5 minutes (mean ± SE). Excretion of N and P was significantly higher in all nutrient-dosed mesocosms than ambient nutrient controls. However, under ambient nutrient levels, gold and copper nanoparticle treatments each significantly increased snail N and P excretion. These results imply that nanoparticles may interfere with snail digestion and nutrient assimilation, particularly under low nutrient conditions.

## Integrating Big Data Into the Bigger Picture

**312 How to develop targeted eDNA sequencing tools for geographical mapping of aquatic species: A case study**

*J. Shaw, CSIRO / Land and Water*

It is not financially or spatially plausible to restore degraded habitat zones along entire river systems, but instead restoration efforts must be appropriately-geographically focused for species or communities of interest. To do this successfully, it is critical that information regarding the specific locations of taxa is obtained. Although current survey tools such as traps and nets provide useful demographic information, they are not ideal for large-scale 'screening' operations, as they typically involve significant time and effort to place nets out, collect them in, and to taxonomically identify captured species. This greatly limits the number of sites that can be searched per funding and time allocations. Further, for endangered species, capture rates are low and only further increase habitat degradation, vulnerability to predation, and detrimental health impacts to individuals. Therefore, alternative rapid and cost effective tools such as Environmental DNA (eDNA) sequencing are valuable for geographically mapping the biodiversity of vast river systems. The Murray–Darling River Basin (MDB) is a river system covering one seventh of mainland Australia, maintaining over 2,520 kilometres of diverse riverine habitats. However, fish species endemic to the MDB have undergone severe population declines due to habitat degradation, pollution, and pressures from alien species. This study resulted in the development of eDNA sequencing-based screening tools for both fish biodiversity and also for species-specific targeting of endangered taxa. We enabled the locations of small fragmented populations to be geographically mapped using these rapid and cost effective tools, and this information will assist with the selection of species-specific target areas for habitat restoration in the MDB.

**313 Multi-level effects of chemical oil dispersant and CEWAF exposures in developing mahi-mahi embryos**

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The Deepwater Horizon (DWH) oil spill released more than 200 million gallons of crude oil into Gulf of Mexico waters in 2010. Nearly 2 million gallons of the chemical dispersant Corexit were deployed as a surfactant to expedite microbial degradation of the oil. The timing of the DWH spill coincided with the spawning season for many commercially and economically important marine fish species, including mahi-mahi (*Coryphaena hippurus*). Thus, early life stage fish were likely exposed to oil, as well as varying mixtures of oil and dispersant. Cardiotoxicity, craniofacial malformations, and reduced swimming performance have been observed following oil exposure in mahi-mahi. Other pathways of oil toxicity have been uncovered via RNASeq, including central nervous system degeneration. Sublethal effects of Corexit exposure alone and in combination with oil (CEWAF) are less studied. To better characterize the impacts of CEWAF on mahi-mahi development, mahi mahi embryos (~6 hpf) were exposed to one of three Corexit concentrations or CEWAF with varying Corexit concentrations for 48 hours. The surviving embryos were collected and transcriptomes assessed with RNASeq. Corexit exposure induced80% across all concentrations. Corexit exposures and controls also formed a tight cluster in a principal component analysis. These data suggest that Corexit exposure alone may not have a significant effect on embryonic development in mahi-mahi. CEWAF preparations induced 3010, 2379, and 2565 differentially expressed genes in low, medium, and high Corexit concentrations, respectively. Percent survival was ~65% in CEWAF preparations, a significant decline compared to controls ($p< 0.05$), but not significantly different than Corexit alone. Importantly, varying Corexit concentration in CEWAF exposures did not significantly affect percent survival. These results indicate that Corexit addition alone may not alter overall mortality at this stage, and it does not greatly impact the number of differentially expressed genes of oil exposed mahi-mahi embryos. Ongoing pathway analyses will determine whether CEWAF altered previous HEWAF predictions based on RNAseq data. This research was made possible by a grant from The Gulf of Mexico Research Initiative. Grant No: SA-1520; Name: Relationship of Effects of Cardiac Outcomes in fish for Validation of Ecological Risk (RECOVER).

**314 Transcriptomics to behavior- What the Deepwater Horizon spill can teach us about fish vision**

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The *Deepwater Horizon* (DWH) oil spill released millions of barrels of oil into the Gulf of Mexico, coinciding with peak spawning periods of ecologically important fish species, such as the mahi-mahi (*Coryphaena hippurus*) and red drum (*Sciaenops ocellatus*). Polycyclic aromatic hydrocarbons (PAHs) present in DWH oil has been shown to cause developmental malformations in fishes, with more pronounced effects during early life stages. Mahi-mahi and red drum embryos were exposed to slick oil and assessed for visual function using transcriptomics, histology, and visually-mediated behavioral assays. The major processes impacted following exposure to environmentally relevant PAH concentrations, as determined through RNAseq and qPCR, were associated with eye formation and phototransduction, with a downregulation of genes important in eye development and function. Using the flicker-fusion principle to monitor an optomotor response and histological analysis, oil-exposed larvae exhibited a reduced optomotor response with a reduction in retinal layers and neuronal connections that play an important role in visual function and image processing. The present study provides evidence that weathered crude oil affects the visual system in developing larval fish, and relates oil-induced transcriptomic effects to histological and behavioral-level endpoints. This research was made possible by a grant from The Gulf of Mexico Research Initiative. Grant No: SA-1520; Name: Relationship of Effects of Cardiac Outcomes in fish for Validation of Ecological Risk (RECOVER).

**315 Linking transcriptomics with metabolomics to assess the mechanism of action of naphthalene sulfonate in the Western clawed frog**

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Naphthalene sulfonic acids are priority chemicals being assessed by the Government of Canada's Chemicals Management Plan that act as dispersants and are components used in industrial lubricants, corrosion inhibitors, and commercial jet fuels. However, little is known about their toxicity to aquatic organisms. Western clawed frogs (*Silurana tropicalis*) were exposed to sand spiked with i) 17 – 1,400 µg/g calcium dinonylnapthalene sulfonate (CaDNS) from egg to larvae and with ii) 25 µg/g CaDNS from egg until the peak of metamorphosis. In the larval experiment, the 100 µg/g CaDNS spiked sand only yielded 14 ng/mL CaDNS in the water column, but yet induced deformities in 98% of the larvae. Transcriptomic analyses revealed over 3,600 genes were differentially expressed in embryos exposed to CaDNS, including biological processes and molecular functions related to cellular structural development. Targeted and untargeted metabolomic analyses (496 analytes) showed a decrease in essential amino acids and metabolites related to muscle movement. There was a decline in the reduced and oxidized forms of glutathione metabolites, which corresponded with a decrease in the transcript level of the genes of the enzymes responsible for converting the two (glutathione reductase and peroxidase). In addition, chronic exposure to CaDNS increased the time to metamorphosis, decreased body size, and decreased gene expression related to thyroid hormones. Combining results from transcriptomics and metabolomics can provide a robust approach to demonstrate the manifestation of the effects of contaminants at multiple levels of organization to work towards developing an adverse outcome pathway.

**316 Seeing the forest through the trees: Integrating multi-'Omic datasets with chemical monitoring data and higher level effects**

*D. Simmons, Denina Simmons Scientific Consulting / Biology*

Currently, collaboration between multiple stakeholders and research teams is necessary for conducting multi-omics that are integrated into environmental monitoring studies. A high level of organization and coordination is required during the experimental design and preparation stage to ensure that all samples are collected appropriately, and that information can be tracked among samples and between different teams with ease. In particular, tissue type, exposure duration/season of field collection, sample storage, and labeling can present challenges not typically encountered within a single 'Omics study or a traditional ecotoxicology study. Post-data acquisition, there are separate statistical challenges involved in the integration of different data types, including data format, scaling, and normalization. Finally, biological interpretation of large environmental datasets is the final bottleneck, and normally requires a high level of expertise in using multiple bioinformatics tools and a sophisticated understanding of environmental effects at different levels of organization in order to ensure that the big picture is captured and does not become obscured by the enormous amount of detail – most of which is caused by simple biological variation. In this presentation, portions of data from three different integrated multi-omics ecotoxicology studies will be shared to illustrate the challenges (and successes) of integrating 'Omics studies with more traditional endpoints.

**317 Ecotoxicological effects of metformin and guanylurea on Japanese medaka (*Oryzias latipes*)**

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In recent years, environmental research has recognized the occurrence and fate of pharmaceuticals in the aquatic environment as an emerging issue in aquatic toxicology. One of the most prevalent contaminants is the type-2 diabetic drug, metformin. Metformin has been measured in the ng-µg/L concentration range in surface waters and wastewater effluent. As >90% of metformin is metabolized into guanylurea during the waste water treatment process, its metabolite guanylurea is found in the µg/L concentration range, thus of major concern. Our recent research shows that male Japanese medaka (*Oryzias latipes*) exposed to metformin and guanylurea from embryo through 28 days post hatch have a significant decrease in length and wet weight when compared to control fish. Metabolomics and proteomics are emerging as an efficient method for understanding sub-lethal effects on organisms by assisting in determining the biochemical mode of action in response to exposure to a particular contaminant. This current study showed significant changes in the metabolome of 28 day old male medaka exposed to metformin and guanylurea, indicating significant dysregulation in fatty acid and lipid metabolism including metabolites associated with cellular energetics and central nervous system health. Additionally, expression of critical genes involved in lipid metabolism were also significantly affected. Proteomic effects on early-life stage medaka exposed to metformin and guanylurea will be explored.

**318 Metabolomic analysis of juvenile Chinook salmon exposed to a mixture of pharmaceuticals and personal care products in the lab and field**

*J. Meador, NOAA Fisheries / Ecotoxicology and Environmental Fish Health Program*

A recent study assessed the occurrence and concentrations of a select number of pharmaceuticals and personal care products (PPCPs) in estuary water, effluent, and juvenile Chinook salmon (*Oncorhynchus tshawytscha*) from one reference estuary and two WWTP-impacted estuaries in Puget Sound, WA, USA. We also conducted a laboratory study with fish dosed for 32 days with 16 of the most common contaminants of emerging concern (CECs) detected in feral fish. Data from a recent study provide support for metabolic disruption based on a limited suite of blood chemistry parameters (Meador et al. 2018). The results from that study were further explored with metabolomics to provide a more complete characterization of metabolic impairment. Metabolomics is the evaluation of small endogenous molecules and products of metabolism such as amino acids, fatty acids, nucleotides, and other important compounds in physiological pathways. These data where subjected to multivariate analysis to test for differences between sites and to explore pathway alteration. A targeted metabolomic approach was used to assess the levels of 219 analytes in replicate plasma samples for fish from the field and the lab study. Additionally, an untargeted metabolomic approach was used to examine for metabolite differences in replicate liver samples from these fish as a separate line of evidence. The untargeted approach identified approximately 2,000 unique molecules and these data support the results obtained with the targeted values for plasma samples. A high percentage of metabolites from both the targeted and untargeted approaches were significantly different between fish from the reference estuary compared to fish from the two WWTP-impacted sites. The two impacted sites exhibited similar responses for a number of compounds and were in agreement when metabolites were expressed as fold-change values compared to reference site fish. The altered metabolomic profile is useful as an early indicator of metabolic stress, even though organismal characteristics (lipid content and condition factor) were not different among sites. Evidence of metabolic disruption was also observed in juvenile Chinook salmon exposed in the laboratory to a limited mixture of CECs for both plasma and liver samples. These metabolomic data allowed us to explore altered or impaired metabolic pathways, which may help identify the most important CECs responsible for the observed responses and possibly mechanisms.

**319 Enhancing the utility of the ECOTOX knowledgebase via ontology-based semantics mapping**

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The US Environmental Protection Agency's Ecotoxicology (ECOTOX) knowledgebase contains more than 30 years of reported single chemical toxicity effects data on aquatic and terrestrial organisms. Approximately 900,000 test results covering more than 11,000 chemicals and 12,000 species are available in ECOTOX. While the database is currently used by many sectors for a variety of purposes, a future goal is to allow for computational modeling of the data to identify novel adverse outcome pathways and networks, and assist in predicting chemical hazard and species sensitivity. One obstacle is that ECOTOX captures the study designs and test results using author-reported descriptions, resulting in more than 4000 codes. Relationships among these codes are often not apparent in the current design (e.g., aryl hydrocarbon hydrolase and cytochrome P450 1A), and some codes are uniquely specific to the study of its derivation (e.g., 3rd generation male). To enhance the query capability of the data within and external to the ECOTOX knowledgebase, and to prepare for future computational functionality, the ECOTOX codes were mapped to existing biological ontology classes. A Java-based lookup tool was developed using the ontology browser BioPortal (https://bioportal.bioontology.org/) REST API to semi-automate the code mapping. This tool was designed to allow for batch processing and to make use of BioPortal's annotator and recommender functions so that all ontological class identifiers relevant to a particular ECOTOX term would be returned and specific ontologies recommended. Using this approach, the majority of the ECOTOX codes were mapped to ontological class identifiers; some terms required multiple identifiers to properly describe them. A set of unmapped terms unique to the ECOTOX database were also identified. Manual curation of the results was also conducted to ensure proper context for the mapped classes.

## Immunoanalytical Technologies: Development and Applications for Environmental Monitoring

**320 VHH antibodies are versatile tools for the analysis of environmental chemicals.**

*N. Vasylieva, UC Davis / Entomology; Z. Li, C. Zhang, D. Li, B. Barnych, University of California, Davis; B.D. Hammock, University of California, Davis / Department of Entomology & UCD Comprehensive Cancer Center*

Bio-recognition elements are the key to affinity based bioanalytical technology. Nanobodies obtained from phage display technology have drawn much attention in recent years due to their promising advantages in gene engineering. Camelid single domain antibodies (sdAb, nanobody or VHH) are increasingly popular due to their small size, high stability, ease of genetic manipulation, and ability for continuous reproducible manufacture. We have shown their use in assays for detection and quantification of pesticides, their metabolites and industrial chemicals that are comparable to polyclonal or monoclonal antibody-based assays. Several examples of nanobody-based immunoassays for chemicals will be presented, as well as their alternative applications for exposure monitoring and environmental analysis.

**321 Development of a Fusion Protein and Its Application in a Highly Sensitive Direct Competitive Fluorescence Enzyme Immunoassay for Detection of 3-PBA**

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Pyrethroids are one of the most largely used insecticide classes in the world. It is important to develop sensitive and rapid analytical techniques for environmental monitoring and assessment of human exposure to these compounds. Because most pyrethroids contain a phenoxybenzyl group and 3-PBA is a human urinary metabolite of pyrethroid insecticides and can be used as a biomarker to monitor human exposure to these pesticides. A rapid and sensitive direct competitive fluorescence enzyme immunoassay (dc-FEIA) for 3-Phenoxybenzoic acid (3-PBA) based on a nanobody (Nb)-alkaline phosphatase (AP) fusion protein was developed. The VHH (variable domain of heavy chain antibody) gene of 3P5ThC12 was subcloned into the expression vector pecan45 containing the AP double-mutant gene. The 3P5ThC12-AP construct was transformed into *Escherichia coli*, and soluble expression in bacteria was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Both the Nb properties and AP enzymatic activity were validated by colorimetric and fluorometric analysis. The 50% inhibitory concentration and the detection limit of the dc-FEIA were 0.082 and 0.008 ng/mL, respectively, with a linear range of 0.015−0.447 ng/mL. This assay was compared with LC−MS/MS, and the results indicated the reliability of Nb-AP fusion protein based dc-FEIA for monitoring 3-PBA in urine.

**322 Development of immunoassays for mushroom toxins**

*C.S. Bever, USDA-ARS; B. Barnych, University of California Davis; L. Stanker, USDA-ARS*

Some of the poisonous and deadly mushrooms contain identified toxic components, such as amatoxins or orellanine. A single mushroom can contain enough toxin to make a person very ill and can be potentially fatal. Our goal is to develop antibodies to these toxins, in an effort to make immunoanalytical methods to detect them. Although the toxin itself is too small to be immunogenic, these small toxins can be covalently linked to larger protein carriers in order to render them immunogenic. Both toxins were linked to carrier proteins using unique conjugation strategies and confirmed by matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS) analysis. Antibodies were raised to both conjugates and characterized by competitive enzyme-linked immunosorbent assay (ELISA). In the case of amatoxins, an immunoassay was developed that could sensitively detect α-, b-, and γ-amanitin down to 1 ppb (ng/mL). In addition, toxin detection from mushroom samples was demonstrated using mushrooms known to contain amatoxins. Further assay characterization and formatting is underway to enable rapid detection of these harmful toxins.

**323 Quantifying amatoxin residues via a nanofibrous portable immunoassay**

*C. Zhao, UC Davis / Agriculture and Environment Chemistry; Y. Si, UC Davis / Department of Textiles and Clothing; B. Pan, UC Davis / Department of Agricultural and Environmental Chemistry; C.S. Bever, USDA; G. Sun, UC Davis / Department of Agricultural and Environmental Chemistry*

Enzyme-linked immunosorbent assay (ELISA) is one of the widely used methods for sensitive and quantitative toxin detection. However, conventional ELISA is instrument-dependent, time-consuming, and complicated-operating, limiting its application in non-laboratory regions. Here, we develop a portable, convenient and high-sensitive biosensor by applying indirect competitive immunoassay on nanofibrous membranes. Taken the advantages of the ultrahigh surface area of nanofibrous membranes, biosensor sensitivity was improved by increasing antibody loading amount. Thus, colorimetric signal form trace analytes can be observed by naked eyes within 30 mins, and quantitative analysis can be further achieved by scanning the membrane with a smartphone. The Limits of detection (LOD) was achieved at 0.1ppb for amatoxin, which is comparable to conventional ELISA using a specialized instrument. Thus, the portable nanofibrous biosensor has the potential to be applied in a wider field.

**324 A utility evaluation of an antibody-based biosensor for detection of 3 – 5 ring Polycyclic Aromatic Hydrocarbons**

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Polycyclic Aromatic Hydrocarbons (PAHs) represent a ubiquitous contaminant in the environment stemming from both petrogenic and pyrogenic sources. PAHs also represent a hazardous portion of complex petrogenic sources and as such are of great interest to regulators during remediation activities. Traditional extraction and quantification of PAHs can be both costly and time consuming requiring large amounts of hazardous solvents and may result in weeks of lag time before a large number of samples can be analyzed. Emerging technologies using monoclonal antibodies capable of binding 3 – 5 ring PAHs may dramatically reduce both cost and time, while subsequently increasing the number of samples that can be run. When combined with fluorescence emission/detection, the biosensor is capable of quantifying total alkyl and parent 3 – 5 ring PAHs in a sample. Additionally, this method requires no sample clean-up or treatment with raw water samples run directly. While unable to differentiate individual molecules, this method can be used to screen potential samples for future analysis, and/or assess the fate and transport of PAHs within contaminated areas. Previous work with this technology has demonstrated the ability to quantify to the sub-ppb level. This method has great potential within industry to analyze and/or monitor samples from complex field conditions. For three years ExxonMobil has been collaborating with the Virginia Institute of Marine Science to evaluate the utility of this antibody as a quantification tool across an array of applications. Previous work has validated the sensor as accurately detecting PAH concentrations from a number of different Water Accommodated Fractions (WAFs) that are used in acute and chronic toxicity tests. Additionally, the biosensor has been used to monitor PAH concentrations from samples taken during a simulated sub-surface oil release at the he National Oil Spill Response Research & Renewable Energy Test Facility (Ohmsett facility, Leonardo NJ). Additional work has been done to evaluate the bioavailable fraction of PAHs in sediment pore water as a more accurate predictor of toxicity as compared to traditional whole sediment concentrations. Here we will discuss some of the past work we have completed with the antibody as it pertains to the environmental fate, transport and toxicity in addition to future opportunities this technology may provide.

**325 Antibody-based biosensor technology as a tool for near real-time quantification of PAH contamination in bivalves**

*K. Prossner, M.A. Unger, G.G. Vadas, Virginia Institute of Marine Science / College of William and Mary / Aquatic Health Sciences*

At sites contaminated with polycyclic aromatic hydrocarbons (PAHs) sessile filter feeders such as bivalves are particularly susceptible to accumulation of these contaminants. During a potential PAH-contamination event such as an oil spill, quick assessment of seafood safety is important to stakeholders and resource managers. Traditional quick response methods such as sensory analysis (e.g. sniff testing) of seafood are often unreliable, non-quantitative and met with distrust by the public. More reliable PAH quantification methods such as conventional GC-MS analyses take several weeks and are expensive. A new, highly sensitive, rapid (minutes) antibody-based biosensor method has been developed to measure total PAH concentrations in individual bivalves using small volume (1-2mL) aqueous samples. Hydrophobic PAHs exist in different phases in the environment (e.g. dissolved aqueous phase and bound-organic phase). At dynamic equilibrium, the concentrations of PAHs in different phases are proportional to each other via partition coefficients, or equilibrium constants. This implies that an unknown concentration of PAHs in one phase can be calculated from another by a simple ratio based on the partition coefficient for the two phases. In this study, bivalves were collected from sites in the Chesapeake Bay with varying levels of PAH contamination and were analyzed by biosensor and conventional GC-MS methods. PAH concentrations in bivalve mantle fluid analyzed by the biosensor correlated well with PAH concentrations analyzed by solvent extracted GC-MS methods indicating that equilibrium within the aqueous and lipid phases in oysters is occurring and whole animal concentrations can be predicted reliably. The biosensor method shows great promise to rapidly and accurately predict PAH concentrations in the bivalve lipid-bound phase based on dissolved PAH concentrations measured in bivalve mantle fluid (e.g. aqueous phase). Biosensor technology could serve as an important tool for rapid evaluation of PAH-contaminated seafood in time-sensitive situations such as oil spill response. This technique is also significantly cheaper than conventional methods on a per sample basis. Further applications may include rapid and economical mapping of background PAH levels in the Chesapeake Bay or Gulf of Mexico oyster reefs to better characterize effects of spill events.

**326 Development of a nanobody and Urchin-like gold nanoparticle immunochromatographic strip test for rapid detection of aflatoxin B1 in grains**

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An immunochromatographic strip (ICS) using urchin-like gold nanoparticles (UGN) and nanobodies for sensitive detection of Aflatoxin $B_1$ ($AFB_1$) was developed to meet the requirement for rapidly monitoring AFB1 in grain samples. The detection limit of $AFB_1$ in a grains matrix was 0.5 ng/mL, which represents a Sixteen-fold increase in sensitivity over conventional Enzyme-linked immunoassay. Analysis of $AFB_1$ in grain samples revealed that data obtained from the ICS were in a good agreement with those obtained from HPLC and ELISA. This qualitative test based on the visual evaluation of results did not require any specialized equipment and the assay time was less than 10 min, which is suitable for rapid on-site testing of $AFB_1$ in grains samples.

**327 Direct and rapid detection of okadaic acid from crude shellfish extracts with a portable multi-channel surface plasmon resonance (SPR)-based biosensor**

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The numerous cases of toxin-associated illnesses from consumption of contaminated shellfish illustrate the need forrapid, regenerable and sensitive detection assays for a wide variety of these aquatic toxins that can be easily used on-site. We describe here a buffered methanolic extraction protocol for the detection of levels of the diarrhetic shellfish toxin okadaic acid (OA) below the guideline levels in shellfish samples using a portable surface plasmon resonance (SPR) sensing system. The gold sensor surfaces were coated with BSA conjugated to OA with the standard EDC/Sulfo-NHS coupling protocol. The assay was run in competitive mode where an analyte-free solution of anti-OA antibodies was run through the system to establish a “no-analyte” rate of antibody binding. The presence of analyte in solution decreased the binding rate proportional to the concentration of analyte. Following each detection event, the bound antibodies were removed by automatically flowing a regeneration buffer (220 mM NaOH/20% acetonitrile) over the sensor surfaces followed by an automatic rinse with running buffer. Reagent preparation involved coupling the antibodies to immunomagnetic nanobeads that allow for a rapid purification and concentration of the toxin from the shellfish matrix. Immuno-nanobeads also increase the SPR signal dramatically over antibody alone. This increase in total signal also increases the sensitivity of detection by approximately 50-fold to a limit of detection below 0.1 ng/ml. This portable detection protocol was designed for ease of use at harvesting sites by injecting diluted extraction buffer or bead washed sample directly into the detector system. Employing the “dilute and shoot” or nanobead approach avoids the more complex evaporation and reconstitution steps. The detection system is capable of multiplexing to include the simultaneous detection of multiple toxins from a single sample injection. In addition to development of the detection assay, recent advancements have improved the performance and portability of the SPR detection sensor including flow cell improvements, lowering power consumption for battery operation, and reduced instrument size for improved portability. These developments combine to produce a portable system for use in on-site toxin detection protocols. The system is also adaptable for use in autonomous multiplex monitoring of several toxins or toxin congeners simultaneously as well as other environmental hazards.

# Chemical Mixtures in Urban Systems – Screening and Prioritization of Emerging Contaminants

### 328 Development of Target Screening Method for 489 Polar Pollutants by LC/QTOF-MS

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Highly efficient analytical methods are necessary to determine the concentrations of the large number of chemicals used in the society that end up in the environment for understanding their potential environmental impacts. Many of these chemicals are best determined using liquid chromatographic mass spectrometric methods (LC/MS). In the present study we developed new target screening method for 489 polar substances, including 298 pesticides and 162 PPCPs, in water samples by LC/QTOF-MS. A water sample (200 ml) spiked with surrogates is first extracted by tandem SPE (Waters HLB-plus and AC-2 cartridges), and then the extract is concentrated to 0.5 ml. Finally, after adding internal standards, the concentrate is screened using a LC/QTOF-MS (Sciex X500R) by positive ESI and Swath. Results of recovery tests using purified water at two concentrations (0.5 and 0.05 ug/L) showed average recoveries of 87.0 and 87.4% (RSD, 9.4 and 9.0%, respectively). Limits of detection of most targets are below 1 ng/L, which is sufficient for environmental analysis. The method was applied to influents and effluents of sewage treatment plants in Japan, with target chemicals identified using retention times, exact precursor and product ions and their ion ratios that are obtained by Swath. Matrix effects that might affect quantification were examined using extracts of influent (100 x concentrated) and effluent (400 x concentrated) and methanol spiked with 213 pesticides (100 ng each). Average ratios of detected amounts between the extracts and the methanol solution were 89.8 (influent) and 91.7% (effluent) with RSDs of 22.6 and 22.6%, respectively, showing that matrix did not affect quantification. The number of substances detected in an influent and an effluent collected in autumn 2017 in Kitakyushu City were 74 and 86, respectively. The detected compounds were mainly PPCPs, but 8 and 16 pesticides were also detected. Total concentrations were 95.6 and 35.8 ug/L, respectively; the removal ratio was 62.9 %. Emission loads of the targets from human activities were 44.5g/1000 capita/d. The data confirmed that this newly developed method is very useful because of the large number of targets, reliable identification and quantification performance. In addition, since the method measures samples by scan, non-target analysis and retrospective analysis can be done using exact mass spectra.

### 329 Pharmaceutical concentrations in Great Lakes tributaries: Chemical and site prioritization based on the ToxCast High Throughput Screening database

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Great Lakes tributary monitoring results during the first six years (2010-2015) of the Great Lakes Restoration Initiative indicated that the level of pharmaceutical chemical contamination in streams have the potential to exert adverse impacts on aquatic life. Subsequently, a more in-depth study was undertaken to examine potential biological effects of pharmaceuticals on fish and wildlife in tributaries of the Great Lakes. Samples from 45 tributary locations were analyzed for pharmaceuticals from an established pharmaceutical method (109 compounds) and preliminary results from a method under development (more than 180 additional compounds under consideration). Samples were collected during 2017 and 2018 to examine variability in pharmaceutical prevalence due to land use, hydrology, and season throughout the Great Lakes region. Sampling locations included very small (< 20 $km^2$) to very large (6330 $km^2$) watersheds with a full range of urban land cover (0-100%) and variable wastewater effluent influence (0-28% of mean annual flow). Relevance of pharmaceutical exposure to aquatic life is being evaluated by comparison to traditional water quality benchmarks and the USGS Health-Based Screening Levels for pharmaceuticals, as well as results from "high throughput" in-vitro biological assay results from the USEPA ToxCast program. Collectively, these sources include available information for about 2/3 of the 289+ pharmaceutical compounds monitored. Results will be used for screening and prioritization of chemicals and sites with the greatest potential for adverse biological impact using Exposure—activity ratios with ToxCast endpoints and toxicity quotients with other benchmarks through the R-package, toxEval. These analyses are being used as part of the Great Lakes Restoration Initiative to focus current and future investigations that will help understand likely adverse outcome pathways in organisms, and to formulate possible remediation strategies. The contents of this abstract neither constitute, nor necessarily reflect, official USEPA policy.

### 330 Effect-based approach for evaluation of brominated dioxins and dioxin-like compounds in effluents and indoor air from BFRs treating facility

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Brominated dioxins, particularly polybrominated dibenzo-*p*-dioxins and dibenzofurans (PBDD/Fs), have been found in various samples from brominated flame retardants (BFRs) treating facilities related to fire-proof materials and electronics in the developed and developing countries. PBDD/Fs are dioxin-like compounds based on criteria of WHO/UNEP expert meeting. Because of that, WHO toxic equivalents (TEQs) should be also determined for PBDD/Fs as well as chlorinated dioxins. On the other hand, some kinds of BFRs currently used may also be dioxin-like compounds according to our unpublished data. Therefore there is a need for effect-based approach by using cell-based assay in combination with instrumental analysis to properly evaluate dioxin-like compounds related to BFRs for their risk management. In this study, in order to aim for an efficient and effective risk management for those compounds, we improved the effect-based method we previously developed for the detection of brominated dioxins, applied this to effluents and indoor air of several facilities related to BFRs in Japan, and considered how to utilize this method in the further investigation. The improved method was resource-saving and time-saving method with high reproducibility and high recovery rate, estimating 2,3,7,8-TCDD-equivalent (TCDD-EQ) of brominated dioxins by using cell-based assay and cleanup/separation technique. And then, both the TCDD-EQ of brominated dioxins fraction ($TCDD\text{-}EQ_{Br\text{-}DXN}$) and the brominated dioxins-derived WHO-TEQ ($WHO\text{-}TEQ_{PBDD/Fs}$) for effluents ($n$=20) and indoor air ($n$=20) were obtained by using the improved method and the GC-HRMS analysis with the WHO toxicity equivalency factor (TEF), respectively. The ratios of the $TCDD\text{-}EQ_{Br\text{-}DXN}$ to the $WHO\text{-}TEQ_{PBDD/Fs}$ was at least 0.1 or more, suggesting that action values should be 10% of their standard limits based on $WHO\text{-}TEQ_{PBDD/Fs}$ when the improved method used as a tool for screening. This means we must steps to analyze PBDD/Fs concentration by using GC-HRMS when action values are exceeded. Furthermore, several samples clearly indicated that ratios were more than 10, suggesting that unidentified brominated dioxin-like compounds may have contributed to the $TCDD\text{-}EQ_{Br\text{-}DXN}$, as mentioned earlier. In conclusion, the method improved here would be useful for an efficient and effective risk management of dioxin-like compounds resulting from the lifecycle of consumer product containing BFRs.

### 331 Removal of Emerging Organic Contaminants in an Engineered Hyporheic Zone using High Resolution Mass Spectrometry

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The hyporheic zone (HZ), the interface of surface water and groundwater exchange in a lotic system, is a natural bioreactor that can attenuate chemical contaminants. Given this attenuation capability, including hyporheic design elements (HDEs) in urban stream restoration projects can provide simultaneous biological health, habitat, water quantity, and water quality improvements. Engineered hyporheic zones can provide the potential to treat non-point source pollution (i.e., urban stormwater) via in-stream treatment, saving space and improving water quality. Here, we characterized the hydrology and water treatment potential of an engineered hyporheic zone in the Thornton Creek Watershed in Seattle, WA during both baseflow and stormflow conditions. Dye and bromide were used to hydraulically link downwelling and upwelling zones and estimate flow path hydraulic retention times (HRTs) to guide water quality sampling. We applied non-target and suspect screening analysis via high resolution mass spectrometry (HRMS) to detect and identify novel emerging contaminants, then evaluated water quality improvements during HZ transport. After data reduction, we prioritized HRMS features for identification using suspect screening and peak area (abundance) filters. We identified ~70 contaminants with confidence level S2b or higher, including 6 families of poly(ethylene glycols), poly(propylene glycols), and ethoxylates. Expanding on previous data analysis strategies, we evaluated the fate of both identified contaminants and non-target features in the HZ, observing an overall water quality improvement in hyporheic flow relative to surface flow. During stormflow, 56-77% of non-target detections were degraded or eliminated via hyporheic flow, with higher contaminant removal efficiencies at longer hydraulic retention times.

### 332 Bisphenols in San Francisco Bay Water

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Bisphenols are synthetic, organic compounds with broad uses including the manufacture of polycarbonate plastics, epoxy resins that line food containers and water pipes, and as a coating on thermal paper, among others. High production volumes have led to the ubiquitous presence of Bisphenol A (BPA), the most commonly produced bisphenol, in the environment. Bisphenol A is an endocrine disruptor and is highly toxic to aquatic life, with a predicted no effect concentration (PNEC) of less than 1 μg/L. Human health concerns have led to bans and phase-outs of some uses of BPA, which in some cases has resulted in increased use of structurally-similar substitutes that are not well-understood with regards to both their toxicity and their presence in the environment. Serving as home to over seven million inhabitants and a breadth of industry, the San Francisco Bay is an excellent location to screen for the presence of bisphenols in the environment. Sixteen bisphenols were measured in ambient water samples collected from 22 Bay sites in 2017. Findings from this study will be presented to support a more robust understanding of the various bisphenol compounds present in waterbodies near urban centers.

### 333 Prioritizing Contaminants in Complex Mixtures using In vitro-based Metabolomics and Multivariate Statistics

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Chemicals occur as complex mixtures in developed watershed streams, and the makeup of these mixtures can differ dramatically based on land use and a variety of other factors. While it is accepted that some chemicals (and chemical mixtures) are detrimental to ecosystem health, there is rarely compelling evidence as to which chemicals, chemical classes, or defined mixtures are the most biologically impactful. To address some of these needs, the USGS and the EPA partnered to conduct an extensive field study of 38 streams across the US during 2012-2014. Water was collected for expanded target analysis (916 total analytes), and split samples were submitted to a variety of effects-based tools for evaluating the biological impacts of chemicals (and other stressors) in these waters. Here, we discuss an untargeted NMR-based metabolomic assessment of these samples, using cultured zebrafish liver cells (ZFL). One goal was to use partial least squares (PLS) regression to determine which stressors most strongly co-varied with changes in metabolite profiles, and conversely, which stressors showed little to no covariance and thus potentially less biological relevance. Of 455 stressors submitted to PLS regression (including 406 anthropogenic organic chemicals detected in at least one site, 37 inorganics, and 12 water quality parameters), 280 displayed significant covariance with impacts on the ZFL metabolome. To provide a coarse ranking, these 280 stressors were sorted into quartiles according to the strength of their covariance. The top (i.e., strongest covariance) quartile comprised a mix of legacy contaminants (e.g., PCBs), contaminants of emerging concern (CECs), and a few inorganics. Also, several non-chemical stressors (e.g., pH) displayed strong covariance, highlighting the ability of this untargeted approach to capture impacts from these types of stressors as well. Interestingly, none of the 10 most frequently detected organics (common pesticides and pharmaceuticals) occurred in the top quartile, and 6 of the 10 were omitted altogether due to minimal covariance. The 10 organics that were detected at the highest median concentration were better represented in the top quartile; however, overall, there was no correlation between occurrence level and ranking within the PLS model. These and other aspects of this work will be presented to showcase the utility of multivariate statistics and in vitro-based metabolomics for prioritizing contaminants in complex mixtures.

### 334 Using predicted estrogenicity to drive High Resolution LCMS non-target chemical identifications in complex environmental samples

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The persistence of consumer product chemicals has been observed in solid and aqueous wastewater treatment plant discharges worldwide. A number of these chemicals has been shown to cause endocrine disruption, for example in organisms downstream from wastewater discharge locations. Sewage sludge is a concentrated, complex mixture of compounds that may ultimately be introduced into the environment via land application practices. Looking for consumer products chemicals, and their transformation products, in sewage sludge offers a bottom-up approach to look for compounds that have persisted beyond their intended period of use and the robust treatment processes employed at wastewater treatment plants. Fourteen wastewater treatment plants in California have contributed end-of-treatment sludge samples for this study. In coupling High Resolution LC-QTOF-ESI-MS non-target screening with predicted estrogenicity data using the VEGA toxicity prediction model, we are able to prioritize chemical identifications of biologically relevant, persistent organic pollutants. Furthermore, in-vitro bioassay data and effects-directed analysis will be used to further analyze the estrogenicity of this complex matrix and attempt to account for cumulative estrogenicity through non-target chemical identifications.

## A Dialogue and Debate on Science, Sustainability and the Science of Sustainability

**336 Cross fertilization between eco-innovation and life cycle assessment: A pathway to circular economy**

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Since the industrial revolution, the use of natural resources and the generation of waste has grown in an accelerated and uncontrolled way, in a process that has led to the so-called ecological crisis. This reality, derived directly from the current economic patterns of exacerbated production and consumption, characterized by the logic of "extract-production-consumption-disposal", which assumes that resources are plentiful, available, and cheap. The circular economy proposal arises as an alternative to this traditional linear approach, proposing a model based on "reuse-repair-renew-recycle" existing materials and products, in order to tackle this serious ecological crisis. Circular economy ideals then proposes that resources are kept in use for as long as possible, thus proposing that inputs and products need to be managed in a more eco-efficient way throughout their entire life cycle. Because of its characteristics, life cycle assessment (LCA) has the potential to play an important role in this paradigm shift not only because of its already recognized potential for evaluating the environmental impacts associated with all the stages of the life cycle of a product but also because of its still little discussed role as a promoter of opportunities and possibilities to eco-innovate. In this sense, for the transition to the circular economy, life cycle approach has shown to be an appropriate and comprehensive tool to support decision-making processes related to environmental issues in business and also for their important characteristic of providing perspectives for eco-innovation. The present work aims, through a review of the recent literature, to analyse the ways for a conceptual approximation of the approaches of these three axes of knowledge: circular economy, eco-innovation and LCA. The discussion takes into consideration and aims to shed light on the cross fertilization between eco-innovation and LCA as well as consider the individual participations of both areas of knowledge, seeking to evidence their potential to contribute to the necessary transition to the circular economy. This review and a logical analysis of the three approaches highlight specific points where the approach is recognized not only as feasible but also advantageous, leading to the conclusion that the path of integrating approaches is promising.

**337 The Science of Sustainability? Scaling the interaction between Resilience and Robustness**

B. Dyson, *T. Canfield, J.F. Carriger, Jr., USEPA / National Risk Management Research Laboratory*

The failure of social-ecological systems to be robust to changing conditions points to a need to better understand ecosystem response and ecological resilience as a guide to ensure sustainability of ecosystem service provision. While a systems-level understanding of resilience may be useful, and potentially necessary, it is not sufficient for defining sustainability requirements e.g. the four questions: what; for whom; how long; at what cost, and relevant decision metrics e.g. Triple Bottom Line, that are needed for localized provision of sustained services. Sustainability of social-ecological systems is a function of the interaction between a larger dynamical system understanding of ecological resilience and more localized decision-making and designing of robust systems for maximizing the sustainability of ecosystem service delivery. Understanding ecosystem resilience characteristics provides a larger context or set of operating principles for incorporating adaptability into the planning and execution of service delivery system. Examples include hybrid approaches such as green infrastructure and decentralized networks for water treatment and waste management. Modeling (system dynamics) and analytical approaches (decision analysis/MCDA) for system characterization, problem formulation, option development and implementation will be discussed.

**338 On the integration of complexity and integrity to restore and sustain the Blackfoot Watershed, Montana**

C. Curtin, *Blackfoot Challenge*

For more than 25 years Montana's place-based collaborative the Blackfoot Challenge has focused on sustaining the cultural and ecological integrity of the Blackfoot River watershed in a 1.7 million portion of the headwaters of the Columbia River. Famed for its depiction in the book and movie "A River Runs Through It" two decades ago the river was severely polluted, many of the key wildlife species were missing from the valley, and wooded uplands were too frequently degraded by logging. Today the watershed is considered one of the healthiest in the US. Top carnivores such as grizzly bear and wolf have returned. We have an overall species diversity comparable to the world renowned Yellowstone National Park, despite seven towns and over 8,000 people being located in the watershed. Much of this success can be attributed to a process that recognizes that managing complexity requires designing for complexity in an open and transparent process. The governance is largely focused on building and sustaining trust and it is an excellent example of how systems science needs to blend social and ecological perspectives through principled approaches. The governance and collaborative stewardship process is a significant example of how getting the science right requires building durable institutions and that in restoring large systems almost everything flows from maintaining and sustaining the social capital that allows collaboratives to "Get Stuff Done." In this talk I use the Blackfoot Challenge example to illustrate how science, conservation, restoration, and culture are entwined and how institutional design is crucial to maintaining large and complex systems. I also focus on how climate, fire, wildlife, and rural economies interact to influence the watershed and how we are redesigning and realigning programs to meet existing realities though a complex systems-based approach.

**339 Four Sustainability Questions and Seven Things**

C. Stahl, *USEPA Region III*

An analysis or discussion of a set of indicators that include some economics, some societal and some environmental factors (i.e., the Triple Bottom Line: Economics, Society and the Environment) are many times considered an adequate addressing of sustainability. The fallacy in this approach is that without the Environment, neither Economy nor Society could exist. When problem formulation about sustainability does not accurately reflect the problem to be addressed, we often solve the wrong problem or exacerbate the problem. Sustainability is a wicked problem (Rittel and Webber, 1973). Therefore, it is important to be explicit and deliberate about how we conduct problem formulation for sustainability. An alternative to this is the concept of the Four Sustainability Questions posed by Allen, Tainter and Hoekstra (2003) in "Supply-side Sustainability." These Four Questions are: 1) What do we want to sustain?, 2) For Whom do we want to sustain it?, 3) For How Long do we want to sustain it?, and 4) At What Cost are we willing to sustain it? Juxtaposed against Patel and Moore's recent book, "A History of the World in Seven Cheap Things," this talk sets the stage for thinking about and acting on sustainability in a fundamentally different way than simply ensuring that the discussion include indicators for economics, society and the environment.

**340 Application of sustainabilty as a value in hard rock mining**

E.J. Dorward-King, *Newmont Mining Corporation / Sustainability and External Relations*

The understanding of and definition of sustainable development or sustainability has evolved over the past decades. This presentation will consider that evolution in the context of the global large scale mining industry and will describe the current application of sustainability by Newmont Mining, where sustainabilty is viewed as a value rather than as a technical imperative with engineering solutions. While application of technical solutions based on scientific data and engineering will be discussed, also included will be the social and economic considerations on which decisions, programs and actions are based. Specific case studies from Australia, Ghana and the USA will be discussed, including approaches and data for economic impact assessments, local economic development, biodiversity and habitat restoration, watershed stewardship, and mine closure planning and implementation. The concept of the triple bottom line as a foundational model for sustainability will be defended.

**341 She Blinded Me With Science ... And Bored Me With Math ... Until I Understood Systems!**

*R. McCormick, Bureau of Land Management*

Yes, it’s sad the polar bears don’t have ice to hunt on, but do the societal math. 9+ billion humans, fixed land base, finite fossil fuel reserves, very low energy ROI for most alternatives to fossil fuels, clear SLR regardless of who or what you want to point the finger at ... these affect every sector of SETAC, every GU, peoples in Europe, native villagers on the Chukchi Sea in Alaska, and Islanders in the Pacific. The math of sustainability and resilience is inexorable, and won’t wait for SETAC members to recognize it. If we were to re-imagine the “Scope of the Meeting” statement on the SETAC website to reflect current sustainability science, it might look like this: The essence of sustainability in practice is to act with increased resilience in mind, and in all aspect of living, working and playing on a social – ecological landscape. The thrust of this meeting is to re-align the importance of sustainable economic development within the lens of ecological and societal stewardship. In this context, stewardship represents the practice of resilience thinking and action towards maintaining and growing a sustainable society. One of the biggest challenges facing society in this endeavor is identifying ways to decouple the historical connection between economic growth and ecological integrity, and the resultant societal effects. To solve this wicked problem, SETAC advocates for evaluating sustainability as a single pillar with economic systems embedded in the societies they emerge from, and in turn the ecological systems in which both reside. This meeting offers opportunities to feature the connections between desired ecosystem goods and services, stable flourishing societies, and sustainable economies. Others in this session will define the single-pillar concept of sustainability. The single-pillar perspective places economies within societies, which in turn are embedded within and wholly dependent on the flow of ecosystem services to the society. If the capacity of the ecological system to provide desired ecosystem services declines, the society must adjust accordingly or experience disruptive societal turbulence. Coping with the ebb and flow of ecosystem services is the key to any society and to its economic status. Discourse in the early days of the 21st Century is disjointed, disagreeable, and dangerous. Holistic, system-based approaches are the only way to overcome this, and science is the process we will use.

## Human Exposure to Emerging Environmental Contaminants

**342 Concentrations of Perfluoroalkyl Substances in Drinking Water, Indoor Air and Dust in Ireland: Implications for Human Exposure**

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This study assesses for the first time, exposure of the Irish population to perfluoroalkyl substances (PFAS) in common indoor microenvironments and drinking water. To do so, passive air sampler (PAS) and floor dust samples were collected from Irish homes, cars, offices and primary school classrooms (n=30 per microenvironment) between August 2016 and January 2017. PAS consisting of a sorbent (XAD-3) impregnated polyurethane foam disk (PUF) were deployed for 60 days, placed on elevated surfaces in homes, offices, and schools, and the floor behind the passenger or driver’s seat in cars. Dust samples were collected by vacuuming floors following methods previously described. In addition, 60 tap water samples were collected from the same homes and offices, with a further two samples of each of five major mineral water brands (i.e. 10 bottled water samples in total). PFAS were detected in all air and dust samples with an average ΣPFAS concentration of 290 pg/m$^3$ (range: 0.03 –2300 pg/m$^3$) and 67 ng/g (range: 0.4 – 4000 ng/g) respectively. In air, PFOA (average: 140 pg/m$^3$, range: < 0.1 – 1200 pg/m$^3$) was the dominant congener in 60% of samples. This was followed by PFBS (average: 38 pg/m$^3$, range: < 0.2 – 310 pg/m$^3$) and PFOS (average: 73 pg/m$^3$, range: < 0.2 – 1600 pg/m$^3$) that were dominant in 21% and 18% respectively. In dust, PFBS (average 16 ng/g, range < 0.1 – 170 ng/g) was the dominant PFAS in 68% of samples. PFOS (average: 27 ng/g, range: < 0.1 – 2700 ng/g) was dominant in 15% of samples, and PFOA (average: 8.1 ng/g, range: < 0.05 – 380 ng/g) was dominant in 8% of samples. PFAS were detected in all bottled water samples and in 86% of tap water samples. The average ΣPFAS concentration in bottled water samples was 16 ng/L (range: 0.17 – 64 ng/L). The average ΣPFAS concentration in tap water was considerably lower at 0.94 ng/L (range: < 0.5 – 16 ng/L). PFBS was the dominant congener in bottled water making up 75% of the average ΣPFAS content followed by PFOS (12%). PFBS was less dominant in tap water (51%) with PFOA making up 30% of ΣPFAS. Our data are consistent with a shift towards the use of lower carbon chain PFAS.

**343 Identifying chemicals in the indoor environment that can serve as tracers for estimating children’s non-dietary ingestion from dust exposure**

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Children are exposed to dust in indoor environments through their regular activities. Dust is known to be a sink for many chemicals from indoor sources such as building materials, furniture, cleaning agents, insecticides and personal care products. Partitioning and accumulation of chemicals in dust contribute to children’s uptake of these chemicals through non-dietary ingestion, inhalation and dermal contact. Dust is routinely collected in observational human exposure studies but its potential to be used as a standard metric for human exposure to chemicals remains unclear due to the large variability in dust composition and human exposure patterns. Previous studies have tried to calculate dust ingestion rates using metals or other earth elements as tracers. These approaches have significant limitations since the proposed tracers are present in matrices besides dust. Our study aims to identify organic chemicals that are unique and ubiquitous in dust, and that can be used as tracers to calculate dust ingestion rates for children. We collected data from three studies that sampled: 1) 50 homes across the U.S., 2) 38 homes in CA and 3) one house in NC with samples collected from each room and at three time-points. Using non-targeted analysis methods, we generated data on thousands of observed dust features, and tentatively identified compounds that were ubiquitous in each sample set (i.e., appearing across homes, rooms, and time points with high detection frequency [DF]). Candidate compounds include tris-(2-chloroisopropyl)phosphate (plastic production, 98% DF); 2-hydroxy-4-methoxybenzophenone (plastic UV stabilizer, 90% DF); fipronil (insecticide, 95% DF) and piperine (black peppercorn, 85% DF), among others. The detected compounds covered a large spectrum of physicochemical properties, from nonpolar and semivolatile to polar and non-volatile compounds. Future work will include applications of statistical and environmental fate modeling to track emission sources and describe the partitioning of the detected chemicals in the indoor environment to better understand their utility as tracers. More details on the candidate compounds and supporting information will be presented.

**344 Targeted and non-targeted analysis of serum exposome using two-dimensional gas chromatography coupled to high resolution mass spectrometry**

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Environmental risk factors, such as diet, smoking and exposure to environmental toxicants, may contribute more than genetic factors to the risks of cancer and other non-communicable diseases. Persistent organic pollutants (POPs) represent an important subset of toxicants that exhibit common characteristics, such as persistence, toxicity and a tendency to bioaccumulate. This contribution reports on a sensitive, quantitative and high-throughput method for the analysis of legacy and emerging halogenated POPs in 200 µL of serum. Serum samples were collected from mothers (n=68) at Mount Sinai Hospital (Toronto). The method employs stir-bar sorptive extraction, which enables preparation of >20 samples in four hours. Detection is achieved using thermal desorption gas chromatography coupled to a quadrupole time-of-flight mass spectrometer. Sensitivity is enhanced by atmospheric pressure gas chromatography, a soft ionization technique that maximizes the yield of molecular ions. A Zoex ZX2 modulator enabled GCxGC separation, which significantly increased the number of detectable chemical features. Data processing and visualization was performed using GCImage HRMS version 2.5. Unknown halogenated compounds were identified using custom macros. The identities of most environmental toxicants and their roles in causing chronic diseases are not known. The full scan data acquired using the qTOF represents a trove of data that can be retrospectively searched for as yet unidentified toxicants. The results of this study indicate that identification of trace level POPs is feasible provided a strategy is implemented to effectively remove interfering chemical features. To accomplish this: (1) a library of chemical features was generated through GCxGC-HRMS analysis of pooled samples obtained from study participants. GCxGC separation proved crucial to obtain clean mass spectra that would otherwise be obscured by matrix; (2) a software filter was devised to separate halogenated from non-halogenated compounds on the basis of accurate mass and isotope ratio measurements; (3) the filter was applied to the pooled samples, generating a suspect list of features which was used to interrogate the samples obtained from individual participants. Preliminary results indicate the presence of multiple classes of POPs that show an inversely proportional correlation to birth weight. These POPs include a number of halogenated chemicals whose identities have not yet been reported.

**345 Urinary concentrations of chlorophenols in pregnant women and children from Sheyang Birth Cohort**

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Chlorophenols (CPs), have been widely applied as raw materials or intermediates for fungicides, insecticides, and wood preservatives for several decades. Epidemiological studies indicated long-term exposure to low level of CPs were associated with potential adverse effects of human health. However, reports of current exposure levels to these chemicals in pregnant women and children are very limited. In this study, a total of 1,100 mothers were included in the Birth Corhort Study from Sheyang County, Jiangsu Province, and 436 3-year-old children were followed up. Questionnaire surveys were conducted to pregnant women and their children considering general information, disease history, lifestyle characteristics and living environment. Five CP compounds, namely, 2,4- dichlorophenol (2,4-DCP), 2,5- dichlorophenol (2,5-DCP), 2,4,5-trichlorophenol (2,4,5-TCP), 2,4,6-trichlorophenol( 2,4,6-TCP) and pentachlorophenol (PCP), were measured in urine by gas chromatography-tandem mass spectrometry (GC-MS-MS). The geometric mean values of urinary 2,4-DCP, 2,5-DCP, 2,4,5-TCP, 2,4,6-TCP and PCP concentrations of pregnant women were 0.80 µg/L, 2.34 µg/L, < LOD, 1.23 µg/L and 0.28 µg/L, respectively. CPs were widely presented in all maternal urine during pregnancy except 2,4,5-TCP with detection rate of 29.6%. Five CPs were correlated each other using Pearson correlation analysis. Multiple linear regression indicated urinary 2,5-DCP concentrations were significantly associated with maternal age during pregnancy ($p< 0.05$). CPs exposure were also significantly associated with sampling seasons ($p< 0.05$). Additionally, geometric mean values of children urinary 2,4-DCP, 2,5-DCP, 2,4,5-TCP, 2,4,6-TCP and PCP concentrations were 2.48 µg/L, 4.28 µg/L, 0.02 µg/L,1.21 µg/L and 0.08 µg/L, respectively. CPs can be widely detected in children urine samples except for 2,4,5-TCP with a detection rate of 53.3%. Pearson correlation analysis indicated urinary 2,4,6-TCP concentrations were related to DCPs and PCP ($p< 0.05$). Significant correlations between maternal CPs exposure and children CPs exposure were not observed. These findings suggested that both pregnant women and their children in study region were widely exposed to five CPs.

**346 Elevated Concentrations of Bisphenols, Benzophenones, and Antimicrobials in Pantyhose Collected from Six Countries**

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Pantyhose is a skin-tight clothing made of synthetic fibers and worn by women in many countries. During the production of fabrics from synthetic fibers, many chemicals are used to enhance the performance and those chemicals can pose a risk of exposure to consumers. Little is known regarding the occurrence of and dermal exposure to chemicals present in pantyhose. In this study, concentrations and profiles of 23 endocrine disrupting chemicals including bisphenols, benzophenones, chlorophenols, parabens, and triclocarban (TCC) were determined in 74 pantyhose samples collected from six countries. Pantyhose samples were analyzed by two extraction methods, one by complete dissolution of the fabric and another by traditional ultrasonic extraction. Dissolution of the fabric in 1,1,1,3,3,3-hexafluoro-2-propanol/chloroform yielded up to 286 times higher concentrations of several target chemicals than the ultrasonic extraction. Bisphenol S (BPS) and bisphenol A (BPA) were found in 100% and 96% of the samples at median concentrations of 1430 and 14.3 ng/g, respectively. Several brands of pantyhose contained BPS, bisphenol F (BPF), benzophenone-1 (BP-1), ethyl-paraben (EtP) and TCC at milligram per gram concentrations. Benzophenone-3 (BP-3), 4-hydroxy benzoic acid (4-HB), methyl- (MeP), and propyl-parabens (PrP) were found in ≥ 85% of the samples, at median concentrations on the order of several tens to hundreds of nanograms per gram fabric. Black pantyhose made in Japan and China with 21-50% Spandex contained the highest concentrations of BPS (2.2 mg/g), BP-1 (2.4 mg/g) and EtP (88 µg/g). Calculated dermal exposure doses to BPS, BP-1 and EtP by women via pantyhose were as high as 45900, 50600 and 1800 pg/kg-bw/day, respectively. Pantyhose is a source of exposure of women to several endocrine disrupting chemicals.

**347 A pilot study in occupational exposure to phthalates and organophosphorus esters among Canadian nail technicians**

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Phthalates and organophosphorus esters (OPEs) are endocrine disrupting chemicals associated with adverse health outcomes such as reproductive disorders and neurodevelopmental toxicity. Nail technicians are potentially exposed to elevated levels of phthalates and OPEs as they are present in personal care products (PCPs) including nail polish and nail removers. Two previous studies have estimated phthalate exposures in nail salons by using stationary levels. However, no studies have measured personal exposure to phthalate and OPEs in nail salons to date. In this pilot study, we report on concentrations and profiles of selected phthalates and OPEs in air at nail salons in Southern Ontario, Canada. Two stationary and three personal air samples were taken during two 8-hour workdays in two nail salons. Air samples were collected using OSHA versatile samplers (PUF/XAD-2/PUF sandwich) with personal SKC pumps at 2 L/min and analysed for eight phthalates and four OPEs by gas chromatography with mass spectrometry (GC-MS). OPEs had the highest concentrations among all compounds measured. All OPEs and half of the phthalates, except BzBP, DnOP, DiNP and DiDP, had detection frequencies of 100%. The concentration profile of these compounds was TCIPP > TPhP > TCEP > TDCIPP > DEHP > DEP > DiBP > DnBP. Levels of target compounds found in personal air samples were 1-4 orders of magnitude higher than those found in stationary air samples. The median concentrations of TCIPP in personal samples were 816 ng/$m^3$. The most abundant phthalate was DEHP with a range of concentrations from 1.3 to 243 ng/$m^3$. Total air concentrations of DEP, DiBP, DnBP, BzBP and DEHP found in stationary samples were four orders of magnitude lower than those reported from US hair and nail salons (Tran et al., 2015) and Vietnamese hair salons (Tran et al., 2018). However, levels of OPEs reported here were around three orders of magnitude higher than those values found in Canadian homes (Yang et al., 2018). In conclusion, this pilot data indicates that nail technicians in Ontario studied here, experience inhalation exposure to phthalates at low levels and OPEs at elevated levels.

**348 Examining Children's Exposure to Organophosphate Esters Using Silicone Wristbands**

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Organophosphate esters (OPEs) are commonly used as flame retardants and plasticizers and are frequently detected in indoor environments. Human exposure to these compounds is widespread, and OPE urinary metabolites are ubiquitously detected in the population. This is of concern for children who often experience higher exposures and are vulnerable to adverse long-term health effects. Recently, silicone wristbands have been used as personal passive samplers to examine exposures to semi-volatile organic compounds (SVOCs). Among adults, OPEs were significantly associated with metabolites from pooled urine samples ($r_s$=0.4-0.7; $p<$ 0.05). Here, we examine the utility of wristbands for assessing children's exposure to 21 OPEs through comparisons to pooled urinary metabolites, house dust, and hand wipes. Children ages 3-6 years and their families were recruited in 2014-16 to participate in a study examining SVOC exposures. Paired hand wipes and dust were collected in the home, and children wore pre-cleaned wristbands (n=74) and provided 3 urine samples (collected over a 48-h period and pooled) in support of the study. All measured OPEs were frequently detected on >85% of bands, which suggests children's exposure is common. Geometric means ranged from 6.1 to 614.8 ng/g band, with triphenyl phosphate (TPHP) having the highest concentration overall. OPEs on the wristbands were positively associated with metabolites from pooled urine samples. In particular, tris(1,3-dichloroisopropyl)phosphate (TDCIPP) on the wristbands was significantly correlated with its corresponding urinary metabolite ($r_s$=0.51, $p<$ 0.01). Isopropylated triarylphosphate esters (ITPs) were also significantly correlated with their metabolite, isopropylphenyl phenyl phosphate (ip-PPP; $r_s$=0.24-0.38; $p<$ 0.05). Average outdoor temperature was positively and significantly associated with TDCIPP on the wristbands ($10^B$=1.05), which reflects a similar trend observed for the urinary metabolite; however, this association was not observed for hand wipes. The magnitude of correlation between OPEs in exposure matrices and paired urinary metabolites for wristbands was roughly equivalent to hand wipes in a few cases and were consistently greater than house dust. These results suggest that wristbands and hand wipes provide an improved measure of individual exposure to OPEs compared to house dust. Due to the ease of sample collection, they hold potential to support large-scale biomonitoring and epidemiological studies.

**349 Investigating human exposures to flame retardants and polycyclic aromatic hydrocarbons using silicone wristbands**

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Human exposure to organic chemicals remains crucial for informing public health. Compared to active samplers, personal passive samplers, have the capacity to represent exposures integrated over a longer time-period, are cost-effective and less-invasive. Although silicone wristbands have recently been used for characterizing personal exposure to a variety of chemicals, information on the exposure represented by a wristband is still limited. We hypothesized that wristbands integrate inhalation and dermal exposure. Wristbands, active and passive personal air samplers, and hand wipes were used to examine the relationship between routes of exposures. Each adult participant (n=10) wore one silicone wristband, one silicone brooch/badge (PDMS) and an OVS active personal air sampler containing a glass fiber filter followed by a PUF/XAD/PUF sandwich for 72 consecutive hours. Hand wipe samples were also collected each time before washing hands during the 72 hour period. Samples were extracted and analysed for 9 polycyclic aromatic hydrocarbons (PAHs) and 13 flame retardants (FRs), including novel brominated flame retardants (NBFRs), polybrominated diphenyl ethers (PBDEs), and organophosphate esters (OPEs) using gas chromatographic mass spectrometry (GC-MS). In most samples, congeners BDE-47, -99 and -209 were the most abundant among PBDEs, EHTBB and DBDPE among NBFRs, TCEP, TCIPP, TPHP among OPEs, and phenanthrene among PAHs. Concentrations of PBDEs, NBFRs, OPEs and PAHs in wristbands ranged from 0.1 to 6 ng/g band, 0.1 to 10 ng/g band, 0.1 to 200 ng/g band, and 0.1 to 20 ng/g band, respectively. Levels measured in wristbands were generally positively correlated with levels in hand wipes and OVS samples summed together ($p<$ 0.01), confirming our hypothesis. We conclude that silicone wristbands are suitable for quantitative assessment of human exposure to a large range of chemicals simultaneously, and this exposure represents both inhalation and dermal contact.

## Emerging Flame Retardants: State-of-the-Art Knowledge and Addressing Research Gaps

### 350 Two decades of environmental research on flame retardants – taking stock

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Two presentations at Dioxin1998 in Stockholm on the discovery of polybrominated diphenylethers (PBDEs) in human milk and in sperm whales are considered as the start of brominated flame retardant (BFR) research in the environment and humans at a global scale. Scientists have successfully demonstrated the negative effects PBDEs and hexabromocyclododecane (HBCD), which has resulted in bans of these FRs in many countries. It would be expected that this lesson on the negative effects of these BFRs, following the problems caused by PCBs, would finally lead to a total stop on the production of BFRs and chlorinated FRs. Unfortunately, the current situation is not at all like that. The total global production of FRs increases every year with ca. 4%, including BFRs, while also chlorinated paraffins are still being produced. Meanwhile phosphorous-based FRs (PFRs) have increasingly been produced. The European research project *ENFIRO* showed that some PFRs may be less harmful for the environment. Although bioaccumulation of these PFRs is less than that of BFRs, some chlorinated PFRs do bioaccumulate. The large number of compounds made available and the mixtures that are being used pose new questions with regard to health effects. With bioaccumulation playing a less important role, exposure routes have shifted from food to indoor air and dust due to leaching of the various FRs from electronic equipment and furniture. Currently, authorities in the US and Europe work on restrictions of harmful FRs. At the same time, industry tries to develop alternatives for the ones likely to be restricted in the near future. Halogenated polymers such as brominated polystyrene belong to the alternatives offered as well as halogen-phosphorus FRs such as tris(trisbromoneopentyl) phosphate, di- and tribromoneopentylalcohol, tris(1,3-dichloroisopropyl) phosphate, chloroalkyl-phosphate blends and many others. Many of these alternatives are not environment-friendly. In the 1950s people had 15 minutes available to escape from a fire in their house before a total flashover. Nowadays, in spite of all FRs, this is 3 minutes. The fire brigade still arrives after ca. 8 minutes. It shows that where many FRs have caused a threat to the environment and human health, they have not been able to increase the safety in our homes. It means that this topic still requires much attention, both from the fire safety side as well as regarding negative aspects for the environment and human health.

### 351 Screening Consumer Products for Flame Retardants

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Flame retardants (FRs) have been added to polyurethane foam, textiles, and other consumer products to meet flammability standards, including California's Technical Bulletin 117 (TB 117). Recent legislation, California Senate Bill 1019 (SB 1019) requires labelling of a product as to whether it does or does not contain added FRs. Samples labelled as not containing FRs were collected and submitted by the Department of Consumer Affairs (DCA). In addition, a second group of samples were collected for a furniture replacement study that included furniture samples pre- and post- SB 1019. Together, over 150 samples were analyzed for added FRs. Energy Dispersive X-Ray Fluorescence (XRF) was used to screen samples for the presence of Bromine (Br), Chlorine (Cl), and Phosphorus (P), key elemental indicators for Brominated Flame Retardants (BFRs) and Organophosphate Flame Retardants (OPFRs). The use of XRF allowed samples to be directly analyzed with limited or no sample preparation, increasing sample throughput and providing data to indicate whether a FR was present at an elevated level and information on the class of FR present. A second screening method using Inductively Coupled Plasma – Optical Emission Spectrometry analysis was performed in parallel to measure the concentration of P. Gas Chromatography – Tandem Mass Spectrometry (GC-MS/MS) was used to measure 17 specific BFRs and OPFRs and demonstrate the utility of the screening techniques. Samples analyzed by GC-MS/MS, were homogenized by cryogenic milling, spiked with labelled surrogates and extracted by three cycles of vortexing and sonication in toluene. Data confirmed the reliability of XRF to rule out BFRs if Br was not detected, as well as OPFRs when Cl and or P were not detected when total FR levels measured by GC-MS/MS were below 500 ppm. XRF was determined to be a rapid and effective screening tool to limit the number of samples requiring quantitative analysis to those that contained total FRs above the 1000 ppm regulatory limit. We also report on the number of mislabeled products that are in violation of SB 1019. A three-year trend post adoption of the SB 1019 labeling requirement shows a decrease in the number of mislabeled products from 30 to 20 percent. The views expressed herein are those of the authors and do not necessarily reflect those of the California Department of Toxic Substances Control.

### 352 Brominated & Organophosphate Flame Retardants along a Sediment Transect Encompassing the Guiyu, China E-waste Recycling Zone

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E-waste recycling using crude techniques releases a complex, yet incompletely characterized mixture of hazardous materials, including flame retardants (FRs), to the environment. Their migration downstream, possibly traced by their pollution profiles, and potential risks also remains undocumented. We examined 26 FRs (18 brominated (BFRs: 12 polybrominated diphenyl ether (PBDE) congeners, plus 6 alternatives) and 8 organophosphate esters (OPEs)) in surficial sediments of the Lian River. Sampling encompassed the river's origin, through the Guiyu e-waste recycling zone, to its mouth, as well as associated tributaries. OPE exceeded BFR concentrations in most sediments, despite their far greater water solubilities. Among OPEs, tris(1-chloro-2-propyl) phosphate dominated upstream, but shifted to triphenyl phosphate in Guiyu and downstream sediments. For PBDEs, Deca-BDE dominated upstream, but Penta-BDE prevailed in Guiyu and at many downstream sites. Among emerging alternative BFRs, decabromodiphenyl ethane dominated upstream, transitioning to 1,2-bis(2,4,6,-tribromophenoxy)ethane in Guiyu sediments. Penta-BDE (BDE-47 + -99, 668-204,000 ng $g^{-1}$, PBDEs 2,280-287,000 ng $g^{-1}$), tetrabromobisphenol A (2,720-41,200 ng $g^{-1}$), 1,2-bis(2,4,6,-tribromophenoxy)ethane (222-9,870 ng $g^{-1}$) and triphenyl phosphate (4,260-1,710,000 ng $g^{-1}$, OPEs 6,010-2,120,000 ng $g^{-1}$) concentrations in Guiyu sediments were among the highest reported in the world to date. The continuing dominance of these e-waste indicative FRs in downstream of Guiyu suggested that FR migration from Guiyu occurred. Hazard quotients >1.0 indicated that the extreme sediment concentrations of individual FRs posed ecological risks in most areas of the Guiyu reach and downstream. Simultaneous exposure to multiple FRs likely increased risks. However, risks may be lower if FRs were associated with strong sorbents, e.g., carbon black from burned debris, hydrophobic polymer fragments, or resided as additives within polymer fragments.

### 353 Organophosphite Antioxidants in Indoor Dust Represent an Indirect Source of Organophosphate Esters

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Organophosphate esters (OPEs) are a group of massively produced and applied additives, whose concentrations in indoor dust are related to human health. Thus, accurate analysis of OPEs in indoor dust is the precondition to estimate their adverse effects on human health. Previous study always paid attention on OPEs from direct sources. In this study, indirect sources of OPEs were discussed. Organic phosphite antioxidants (OPAs) are among the most frequently used antioxidants in various household and industrial products to retard oxidation degradation. The OPAs can be oxidized to the corresponding OPEs during the antioxidant process. In this study, five OPAs were detected in indoor dust samples, with geometric mean (GM) concentrations of 2.46-70.4 ng/g. The corresponding OPEs were detected at much higher GM concentrations (30.5-3759 ng/g). Among the detected OPEs, triisodecyl phosphate (TiDeP), trisnonylphenol phosphate (TNPP), tris(2,4-di-*tert*-butylphenyl) phosphate (AO168=O), and bis(2,4-di-*tert*-butylphenyl) pentaerythritol diphosphate (AO626=$O_2$) were the oxidation products of the corresponding OPAs, which represents the indirect source of OPEs. The concentrations of the above four novel OPEs (GM: 30.5-3759 ng/g) are even higher than the well-known tris(2-chloroethyl) phosphate (TCEP), triphenyl phosphate (TPP), 2-ethylhexyl diphenyl phosphate (EHDPP), tris(2-ethylhexyl) phosphate (TEHP) (GM: 111-1827 ng/g), which are mainly from direct source. The present study demonstrates that people are exposed to much more OPEs than we previously knew in indoor environments. To our knowledge, this is the first time to report the occurrence of TiDeP, AO626=$O_2$, and TNPP in indoor dust.

**354 Emerging and legacy flame retardants in plasma of pregnant women from a Chinese preterm birth cohort**

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Preterm birth represents a significant public health concern. Among the variety of etiology which remains unknown in many cases, exposure to environmental endocrine disruptors represents a potential risk, but receives less investigations. Flame retardants (FRs) are a group of chemicals broadly added to various household products and some of them (e.g., polybrominated diphenyl ethers or PBDEs) have already been demonstrated to be persistent, bioaccumulative, and toxic. Previous research has discovered the presence of FRs in humans and the associations with a variety of health effects (e.g. thyroid hormone and androgen abnormalities). This study aimed to determine the concentrations and compositions a wide range of FRs in plasma collected during the first or second trimester of pregnancy women. Participants were from a prospective cohort of 4229 women between December 2009 and December 2012 with 147 cases and 381 controls in North China. Up to 70 flame retardant chemicals, including PBDEs, novel brominated flame retardants, dechlorane related compounds, and organophosphorus flame retardants, were analyzed by using a highly sensitive and selective analytical method. Although the study is still under way, our preliminary results revealed the frequent detection of some emerging and legacy flame retardants (e.g. BDE209, decabromodiphenylethane, tris(chloroethyl) phosphate) in plasma. The results of this study will be employed in the near future to further investigate the association of exposure to flame retardants with the odds of preterm birth risks, as well as the influencing factors, in the study cohort.

**355 Quantitative method development of newly discovered highly brominated flame retardants in Great Lakes Herring Gull regurgitant and terrestrial sources**

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Recent reviews and observations have suggested that one trend for replacement brominated organic flame retardants (OFRs) is towards more highly brominated chemicals with higher molecular weights, such as polymer-like polyhalogenated polyphenol ethers (PHPEs). Although large, highly brominated OFRs possess low bioavailability, it has become increasingly evident that photochemical and/or microbial degradation processes can result in the formation of e.g. lower brominated and more bioavailable compounds. Tetradecabromo-1,4-diphenoxybenzene (TeDB-DiPhOBz) is a PHPE with similar molecular structure to BDE-209 and decabromodiphenylethane (DBDPE), and was the principal component of the now phased out SAYTEX 120 FR mixture. TeDB-DiPhOBz is highly sensitive to photodegradation, resulting in the formation of lower brominated DiPhOBzs and polydibenzofurans. These mixtures of degradation by-products have subsequently been shown to induce CYP1A4/5 mRNA in a concentration-dependent manner in vitro in chicken embryonic hepatocytes. Concurrently, methoxylated polybrominated diphenoxybenzenes (MeO-PB-DiPhOBzs) have been reported in Great Lakes Herring Gull (GLHG) eggs from several colony sites and from collections going back over 40 years. Strong evidence indicates that TeDB-DiPhOBz and its debrominated degradation by-products are absent from typical prey fish of GLHGs, and thus aquatic food web exposure appears to be a minor exposure route. As a result, it is hypothesized that the gulls are exposed via their terrestrial diet component. To date, we are the only lab to investigate TeDB-DiPhOBz in an environmental context. Moreover, to our knowledge there have yet to be any published methods investigating OFRs in avian regurgitant (vomit). The current study adapted and validated existing OFR methods for the quantitative determination of TeDB-DiPhOBz and its photodegradation products via LC-/GC-MS, utilizing newly acquired authentic and pure polybrominated-diphenoxybenzene (PB-DiPhOBz) and MeO-PB-DiPhOBz congener standards on three different and novel sample matrices: herring gull regurgitant, the soil surrounding selected nest areas, and earthworms, from Channel-Shelter Island, Saginaw Bay, Lake Huron. Analyses indicate that debrominated PB-DiPhOBz congeners, but not TeDB-DiPhOBz, were detectable in some of the terrestrial samples. Herring gulls appear to be exposed to TeDB-DiPhOBz via their terrestrial diet but in the form of their PB-DiPhOBz degradation by-products.

**356 Transport of sediment borne emerging flame retardants in a Mediterranean river during a high flow event**

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Pollutants' dynamics in rivers flowing through industrial areas is linked to the transport of contaminants attached to solid particles. The transport of sediment is mainly associated to high discharges and flood episodes, and these events constitute one of the main factors causing fluxes of buried pollutants in rivers. We performed a field study in the lower River Cinca (Ebro basin, Northeast Spain) to quantify the mobilization and transfer of several contaminants present in the river bed sediments. We focused on legacy POPs such as PCBs, DDXs, PBDEs and HBCDs, but also on emerging flame retardants such as decabromodiphenyl ethane (DBDPE) and organophosphate flame retardants (OPFRs). Static sediment samples were collected and analyzed. Both, legacy and emerging pollutants were detected, but the highest concentrations were found for DBDPE and OPFRs, with concentration values ranging from 7.81-121 and 18.8-127 ng/g dw, respectively. Among the different OPFRs analyzed, the highest levels corresponded to tributyl phosphate (TBP), tris(chloroisopropyl) phosphate (TClPP) and triphenyl phosphate (TPHP). A subsequent sampling campaign was used to capture sediment borne contaminants during a flood event. Water samples were taken at the same locations as the static sediments and used to determine the suspended sediment concentrations and the contaminants content (i.e. mass of contaminant per sediment mass unit) during the event. We estimated mass fluxes for both sediment and pollutants, and determined that sediment transport followed a clockwise hysteresis. This is typically observed in high flow events after dry summer periods. With sediments there was a large mobilization of OPFRs (36 kg in 48 h in one of the main tributaries) and PCBs not previously observed in the static sediment. Observed contaminant load ranges during the two-day sampling campaign were: PCB (34–152 g), DDT (12–213 g), PBDE (50–1740 g), HBCD (0–2.2 g) and OPFR (2410–35,895 g). An environmental risk assessment was carried out by comparing pollutant concentrations found in the sediments with the Canadian quality guidelines (ISQC), showing a significant noncompliance for PCBs in dynamic sediments. Our results point out to the need to include emerging flame retardants in river basin monitoring studies, as well as the need of a regular assessment of the downstream transfer of the sediment-borne pollutants in drainage basins historically affected by intense industrial activities.

**357 Flame Retardant Benefit and Harm: Past, Present, and Future**

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Flammability regulations for furniture and foam plastic building insulation were established in North America in the 1970s in an attempt to reduce fire deaths and losses. Flame retardant (FR) chemicals have been used to meet these regulations, but their use has been found to be questionable based on a lack of proven fire safety benefit; potential for FR migration from products resulting in human and ecosystem exposure and adverse health effects. For major product categories, we will discuss the current status of flammability standards driving the use of FRs and whether on not they provide a fire safety benefit to balance their adverse impacts. Upholstered Furniture had metthe California furniture flammability standard Technical Bulletin 117 (TB117) since 1975, which led to FRs in upholstered furniture foam. In 2013, California updated their standard to TB117-2013, which increased fire safety from smoldering ignitions without the need for FRs. In 2017, the US Consumer Product Safety Commission approved a petition to ban the entire class of organohalogen FRs in upholstered furniture and three other products classes. Several US cities and states are banning all FRs in furniture, juvenile products, and/or mattresses. Foam Plastic Building Insulation materials are affordable and energy efficient, but flammable. US and Canadian building codes lead to the use of FRs in such insulation, but they do not improve fire performance of insulation below grade or within a wall cavity behind a thermal barrier. Norway has updated their flammability codes so that FRs are no longer needed in such applications. Similar changes are being considered in the U.S. Electronics suchas televisions cases are one of the biggest sources of FR exposure in our homes with FR concentrations around 25% of the weight of the plastic. A new international fire safety standard allows the redesign of TVs and other electronics so that cases are fire safe without the need for FRs. Conclusion: New policy decisions and regulations based on toxicological and fire science research are considering both fire safety and the health and ecological impacts of FRs. This approach is reducing exposure to harmful FRs. However, in some cases organophosphates FRs are being used instead of organohalogens—a likely “regrettable substitution.” Improving flammability standards is a better way to reduce the unneeded use of FRs compared to moving to the use of replacement FRs which may also prove to be harmful.

## Canadian Oil Sands: Advancing Science in Chemical and Toxicological Characterization, Reclamation and Monitoring

**358 Nontarget screening and characterization of polar polycyclic aromatic hydrocarbons in samples from the Athabasca Oil Sands using LC-Orbitrap-MS**

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Polycyclic aromatic compounds (PACs) are a group of organic contaminants associated with the Athabasca Oil Sands region (AOS) in Alberta, Canada, although more work is needed to understand the relative contributions of various industrial sources to levels of these compounds to the environment s. Furthermore, even though PACs exist in complex mixtures, environmental monitoring in the AOS is often focused on a limited class of, mostly unsubstituted and alkylated PACs, and may fail to account for the more polar PACs that can be as, if not more, toxic. In this study, a nontarget screening approach using liquid chromatography HR-Orbitrap mass spectrometry was used to identify and characterize the distributions of polar N, S, O-containing PACs in snow, air, sediment, and source materials (i.e. petroleum coke, unprocessed oil sands ores, and haul road dust) from the AOS. Because of the excessive data generated from HR-Orbitrap analysis, steps were taken to prioritize PAC-characteristic compounds from the thousands of other peaks detected. Elemental compositions were determined based on accurate mass (< 5ppm) and isotopic abundances. Peaks were refined if they had relative double bond equivalencies between 6 and 18, elemental compositions containing 6< C< 30, and at most 2 S/O/N atoms. Kendrick mass defect plots (KMDs) and MS/MS were used to support and further refine elemental composition assignments. Additionally, KMD plots were able to provide a “fingerprint” profile, showing distinctions between the source samples, and similarities between pet coke and the environmental near-field samples, suggesting pet coke is a potentially important source. Relative distributions based on the normalized areas of detected peaks also confirmed pet coke as the source, as there were similarities in distribution between the pet coke and the environmental samples near major developmental areas. Over 25 “families” (elemental compositions) of polar PACs were detected, including “families” of nitrogen and sulphur-containing PACs previously detected in AOS environmental samples using GCxGC/ToF-MS. This study suggests that further work is needed in characterizing these compounds in the AOS, and that the orbitrap is a useful technique in providing a comprehensive screening and profiling of these compounds.

**359 What does an Athabasca Oil Sands tailings pond plume look like?**

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What is the chemical fingerprint of Athabasca Oil Sands Industry tailings pond waters (TPW) that have escaped into surrounding environments? Many studies and most industry monitoring have used chloride in particular, but also boron and to a lesser extent naphthenic acids (NAs), as conservative ion tracers to infer TPW input. A few have successfully used advanced isotopic data. However, most studies generally lack sufficient detail to infer specific pathway(s) or defined input structure(s). The Environmental Geoscience Program of the Geological Survey of Canada has erected a detailed (38 well and drive points) dataset of hydrology and geochemistry (elemental, organic and isotopic) on a small (100 m by 160 m) test site adjacent to the Muskeg River and down-hydraulic-gradient from a large tailings pond. Along the southwestern boundary of this site is a subsurface conglomeratic aquifer that hosts waters having $d^2H$ and $d^7Li$ isotopic signatures indicating a TPW origin. Aquifer waters have concentrations of B and Li that are 5X to 10X background values, concentrations that are consistent only with a TPW source. Element concentration distributions indicate the aquifer is confined at depth. However, at the southwestern edge of the study area these waters surface and spread across the adjacent wetland, resulting in atypical wetland vertical profiles having higher concentrations at the surface than at depth. The distributions of Be, Cr, F, Rb, V, U, Zr are also consistent with an introduction into subsurface and surface waters via this aquifer. Conversely, chloride and sodium increase away from the aquifer; their molar ratio and distribution demark these as predominantly reflecting road salt inputs. In comparison to TPW concentrations, the aquifer waters are highly diminished (>5X for conservative ions; complete attenuation for many transition metals). Within the site, the aquifer-introduced elements demonstrate considerable and variable attenuation along the flow path (3X to full attenuation); a product of both dilution and sorption as B-normalised contours demonstrate. The combined attenuation profiles produce element concentrations below CCME guidelines throughout the site. The TPW chemical fingerprint has not been detected in the Muskeg River itself, suggesting that the aquifer flux is relatively small.

**360 Temporal variation and source apportionment of snowpack multi-elements deposition in the Athabasca Oil Sands Region**

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Atmospheric deposition of metals in snowpacks and their release into freshwaters during spring snowmelt has been a concern in the Athabasca Oil Sands Region of Alberta. This study was designed to evaluate the concentrations, loadings, and distribution of metals in springtime snowpack and how they vary over time. For a few metals (Al, V) we investigate temporal data as early as 1978 and into 2016. For the remaining suite of metals, we examine several years of data (2008-2016) collected through a rigorous monitoring program, the Joint Oil Sands Monitoring (JOSM) program. Snowpack samples were collected in late winters at varying distances from the main developments. For data from 1978 and 1981 to be comparable to newer data, all metal loads were adjusted to the same number of snow days. Temporal trends were divided into sites less than 8 km from a major industrial site (AR6), 8-50 km from AR6, 50-100 km from AR6 , and reference sites >100 km northeast of AR6. Results indicate that deposition of metals generally decreased over time. Source apportionment indicates that AR6 appears to be the main industrial source for V, but smaller impact of other sources was evident for other elements. Further monitoring is required to ensure that the impact of metals is understood.

**361 Effects of Contaminated Snowmelt from the Athabasca Oil Sands on the Growth, Development and Behaviour of Larval Wood Frogs (Lithobates sylvatica)**

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Oil sands operations in northern Alberta, Canada, are major emitters of atmospheric pollutants, including polycyclic aromatic compounds (PACs) and heavy metals. Previous studies have documented elevated levels of atmospheric pollutants in snowpack within a 50-km radius of two major bitumen upgrading facilities. Amphibians inhabiting wetlands in northern Alberta during spring may be at risk of exposure to pulses of contaminants from melting snow. The goal of our study was to assess the effects of snow contaminated by oil sands operations on the growth, development, and behaviour of a common amphibian species, the wood frog (*Lithobates sylvaticus*). We conducted an outdoor mesocosm experiment at the Queen's University Biological Station in which wood frog embryos were exposed to snow collected from the Athabasca Oil sands region. Snow was collected from three impacted sites (< 50 km from upgraders) and three reference sites (> 83 km from upgraders). Snowmelt treatments were compared to a negative control (natural pond water) and an osmotic control (natural pond water diluted with distilled water). Each of the six snowmelt treatments and the two controls were replicated three times. Wood frog tadpoles were exposed to snowmelt for 25 days, and then were transferred to a clean water environment for an additional 55 days, to simulate the flushing effects of spring rains. Each replicate contained ~250 embryos in the exposure phase and 20 tadpoles during the clean growth phase. We measured tissue PAC concentrations, malformations, expression levels for several genes related to development, body length, body weight, developmental stages and growth rates. Concentrations of PACs in wood frog tadpoles after the 25-day exposure to snowmelt ranged from 272.186 to 2.270 mg/L in the impacted sites and 2.385 to 2.640 mg/L in the reference sites. During the exposure period, tadpoles exposed to snowmelt from impacted sites (0-km and 6-km) showed decreased average body lengths and an increased presence of morphological malformations (gut coiling and body shape). Contrarily, at the end of the clean growth phase, body lengths, body weights and developmental rates of tadpoles from snowmelt treatments were similar to those from the controls.

**362 Sediment-bound dilbit: Comparing direct exposure to the water-soluble fraction on the health of freshwater amphipods (*Hyalella azteca*)**

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The Alberta oil sands contain over 165 billion barrels of bitumen. Bitumen is transported as diluted bitumen (dilbit) after the addition of natural gas condensates. Dilbit is transported predominantly through pipelines, which come in close proximity to freshwater ecosystems. When dilbit is spilled into or near an aquatic environment, much of the volatile added diluents will evaporate, increasing the density of the dilbit. The remaining weathered dilbit has the potential to combine with fine sediment in the water column and sink permanently, affecting different organisms than if it were to remain on the surface. Weathered sediment-bound dilbit (WSD) can be physically toxic to freshwater organisms by coating them or chemically toxic by releasing water-soluble toxicants such as polycyclic aromatic hydrocarbons and their alkylated counterparts. To date, research has focused on the water-soluble and water-accommodated fractions of fresh dilbit and its effects on aquatic organisms. This is the first study that assesses the toxicity of WSD to an aquatic organism. Substrates were formulated that were 100, 50, 25, 12.5 and 6.25% WSD by volume. Adult freshwater amphipods (*Hyalella azteca*) were exposed to these concentrations of WSD for 96 hours. In all concentrations of WSD, there was 0% survival. Additional exposures have shown that the water-soluble fraction of WSD does not negatively affect survival during acute exposure. Ongoing research is addressing if the water-soluble fraction of WSD will alter amphipods physiologically by inducing acetylcholinesterase inhibition, oxidative stress or by altering oxygen consumption. Behavioural experiments are also being conducted to determine if the water-soluble fraction of WSD can change the overall activity of amphipods as a result of these physiological changes. This study shows that different fractions of dilbit may have varying effects on freshwater organisms, which will help to inform for better cleanup practices after future dilbit spills.

**363 PAH concentrations and composition in predatory and forage fish in the Fort McMurray oil sands area with comparisons to environmental regime**

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The Fort McMurray region is rich in natural sources of polycyclic aromatic hydrocarbons (PACs) from exposed bitumen beds; anthropogenic sources have increased with oil sands industry expansion and PACs have been monitored and investigated in several environmental compartments. Here we present highlights of our broad survey of PACs in four species of forage and predatory fish investigated as part of fish health investigations conducted as part of the Regional Aquatics Monitoring Program (RAMP) and the Joint Oil Sands Monitoring program. We also compare fish PAC concentrations and composition with that observed in sediment

and water samples collected as part of these monitoring programs. PAC concentrations in fish, water and sediments were highest at those downstream tributary mouths which flowed through the exposed McMurray formation and along the Athabasca River. ΣPACs averaged 102±32 ng/g (68± 5% alkylated) in trout-perch from the Athabasca River; 278±267 ng/g (75± 13% alkylated) in slimy sculpin from the Steepbank, Firebag, and Dunkirk Rivers; and 125±22 ng/g (64±6% alkylated) in lake chub from the Ells River. ΣPACs concentrations were lower in walleye from the Athabasca River (18±21.6 ng/g, 60±24% alkylated) with still lower concentrations in walleye from Lake Athabasca (1±1 ng/g, 31±27% alkylated) and the Peace River (7±6 ng/g, 65±13% alkylated). Fish ΣPACs were dominated by low-molecular weight compounds, particularly naphthalenes and fluorenes whereas medium and heavy PACs such as dibenzothiophenes, fluoranthenes, pyrene and chrysene, common in sediments, were minor contributors. Phenanthrenes increased in percent composition in fish caught in areas where PACs concentrations in sediments were higher due to the proximity of bitumen sources; dibenzothiophenes, a major component of bitumen PAC, were a minor component of fish PACs. Fish PACs concentrations were below the European Commission (2011) smoked fish consumption guideline of 2.0 ng/g for benzo(a)pyrene and 12 ng/g for the sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene, and chrysene. Fish PAC concentrations also were below guidelines established for the reopening of the commercial fisheries closed by the Deepwater Horizon oil spill. PAC concentrations in fish were similar to concentrations observed in other studies including fish market surveys, estuaries, and marine waters and lower than in highly impacted areas near refineries and other anthropogenic sources of PACs.

### 364 Effects Directed Analysis of Soluble Organics in Bitumen Influenced Waters

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Identification of the organic compounds of concern within bitumen-influenced waters is critical for effective monitoring and reclamation within Canada's oil sands region. Effects directed analysis provides an effective tool to prioritize bioactive and unique compounds of interest in industrial and natural bitumen-influenced waters, allowing for chemical and toxicological characterization of less complex mixtures relative to unaltered waters. Recently identified bioactive fractions from bitumen-influenced groundwater sources were further fractioned using HPLC. The isolated secondary fractions were analyzed using an LC/QToF- MS, indicating successful fractionation with decreasing polarity with subsequent fractions. Toxicity was assessed using the Microtox® assay and *Hyallela azteca*; these assays were selected due to previous responses observed using the primary groundwater fractions. Assays with *H. azteca* required the development of a reduced volume test protocol due to limited secondary fraction volume. The Microtox® and *H. azteca* assays were sensitive to the most polar of the secondary fractions isolated from the most polar primary fractions, further indicating that toxicity is at least in part being driven by the most polar chemical constituents.

### 365 Process-Affected Water in the Oil Sands Industry: Toxicity Attribution and Evaluating Ageing as a Remediation Strategy

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Oil sands process-affected water (OSPW) is used to separate bitumen from sand in the surface-mining oil sands industry of Alberta. OSPW contains a complex dissolved organic mixture that can be toxic, persistent, and is largely uncharacterized. One remediation strategy consists of ageing OSPW in end-pit lakes such that sedimentation and biodegradation of the organics through ageing will eventually allow for safe environmental integration. Predicting the effectiveness of this strategy relies on an understanding of what chemicals cause toxicity in fresh OSPW and how these may change over time. This investigation used chemical fractionation and high resolution orbitrap mass spectrometry combined with cytotoxicity and endocrine disruption assays to further study the toxicity of candidate chemical classes in various fresh and aged OSPW samples. Real-time cell analysis of human liver carcinoma cells (HepG2) and the yeast estrogenic/androgenic screens were used. An isolated fraction containing naphthenic acids (NAs) was largely responsible for the cytotoxicity observed, while the non-acidic fraction, speculated to contain steroidal chemicals, was not cytotoxic. Estrogen/androgen receptor antagonists were identified in all the fractions, with both NAs and the non-acidic fractions active near environmentally relevant concentrations, though chemical antagonism is known to exist within the mixture. Cytotoxicity of OSPW in an end-pit lake decreased as the sample was further aged. However, an even older sample of 23 years that is geographically-separate from the end-pit lake had a unique biphasic toxicity profile, where the organics were initially more cytotoxic than the other OSPW samples tested around 24 hours' post exposure, but then the cells recuperated to close to negative control around 60 hours' post exposure. Therefore, cytotoxicity of an end-pit lake decreased with ageing, which is promising for the remediation strategy, but the biphasic toxicity profile from the most recalcitrant organics should be explored. Furthermore, while NAs were responsible for the cytotoxic effect, the presence of estrogen/androgen receptor antagonists among all the fractions indicate that multiple chemical classes may have the potential for sub-lethal effects on the endocrine system. By identifying the most active chemicals in OSPW there can be a better understanding of observed toxic effects and allow for monitoring to ensure releases are protective of downstream uses.

## Ecotoxicology of Amphibians and Reptiles

### 366 A generalized lizard population model to investigate the impact of toxicity on reptile population dynamics

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There is an interest in putting toxicity data into a population context, with some authors suggesting that population growth rate (or lambda) is an equally important endpoint to consider in ecotoxicology along with effects on survival and reproduction. For the purposes of screening level risk assessment, it would be very helpful to have a generalized population model into which toxicity data can be incorporated to understand the long-term implications of repeated toxicity events. We report on a generalized deterministic lizard population model for *Sceloporus sp* parameterized from demographic data collected from the literature. Our model is a simple 2 stage model with a juvenile stage (1 year) and an adult stage. Because there is so little toxicity data available for reptiles and even less data on their exposure to contaminants in the field, we performed theoretical simulations of 0 to 100% toxicity to each stage of the model (clutch size, juvenile survival, adult survival). We quantified the effects of toxicity on lambda and elasticity values for each stage. The baseline model has a stable population with lambda = 1.07. The fecundity and juvenile survival parameters had the greatest effect on population dynamics. When we applied simulated reductions in survival and clutch size all models showed a decrease in lambda with increasing toxicity. A 20% yearly reduction in juvenile survival or clutch size resulted in lambda decreasing below 1.0 into a slight population decline, while a 30% reduction was needed in adult survival for the same result. Lambda generally was reduced more when juvenile survival or clutch size was impacted. Elasticities changed significantly only when there was high toxicity to juvenile and fecundity estimates (> 60% yearly mortality) at which point adult survival became the most important parameter in the model. Our model suggests that reptile populations are robust to a slight decrease in yearly survival, but repeated reductions in survival will threaten population persistence. This highlights the importance of both quantifying toxicity to these taxa, but also understanding how often exposures occur and if there is any risk of mortality from those exposures. Next steps for the model are shifting the model from a deterministic model to a stochastic model incorporating parameter variation which could be useful for a higher tier risk assessment when risk is found at a lower tier.

**367 Exposure and Effects of common perfluoroalkyl substances (PFASs) on Brown Anoles (*Anolis Sagrei*)**

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Per-and polyfluoroalkyl substances (PFASs) are persistent and abundant environmental contaminants, creating concern about their potential ecotoxicological effects on wildlife. The goal of this research was to investigate the impacts of two specific PFASs, perfluorooctanesulfonic acid (PFOS) and perfluorohexane sulfonate (PFHxS), on a model reptilian species. These PFASs were key ingredients in aqueous film forming foam which was widley used for fire suppression at military installations. Fire suppression activities and use in many commercial products have lead to releases of these chemicals into the environment where wildlife can be exposed. While there is some data on the effects of a small subset of PFASs on common ecological receptors, there is no data available on the toxicity of PFOS or PFHxS to reptilian species precluding a robust assessment of risk to species in this taxa. To address this data gap, we have begun developing brown anoles (*Anolis Sagrei*) as a viable laboratory model as they are abundant, non-native species in the U.S. and amenable to laboratory conditions. We first dosed lizards with PFOS using a pseudo-gavage method three times each week for a total of 35 days. A total of 40 lizards were used with 10 animals per dose which were 0, 0.02, 0.2, or 2 mg/kg per day. Dosing volumes were adjusted based on the weekly weight of each lizard. There were no lizard deaths during the 35 day study but there were significant dose-related effects on growth. All lizards were also necropsied and organs weighed. We found that as PFOS concentration increased, spleen and kidney masses increased. To our surprise, lizards in this study appeared to be similarly sensitive compared to avian receptors that had been exposed to PFOS in a reproductive study. We have subsequently initiated a PFOS exposure study in which lizards have been exposed for 30, 60 or 90 days to PFOS-contaminated sand. The goal is to determine whether dermal exposure for PFOS is a relevant exposure pathway. We have also initiated a pseudo-gavage study to determine whether PFHxS is toxic to lizards at high-end environmentally relevant concentrations. We anticipate that the data developed from these studies will facilitate ecological risk assessments focused on the risk of PFASs to reptilian receptors in PFAS-contaminated habitats.

**368 Assessing the effects of per- and polyfluoroalkyl substances on larval amphibians**

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Per- and polyfluoroalkyl substances (PFASs) are globally distributed contaminants of emerging concern due to their persistence in the environment, accumulation in humans and wildlife, and ability to disrupt endocrine function. The risk posed by these compounds is not well characterized for most wildlife including amphibians. Using a series of laboratory experiments, we examined accumulation and toxicity of perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS), and 6:2 fluorotelomer sulfonate (6:2 FTS) to larvae of the American toad, *Anaxyrus americanus*, northern leopard frog, *Lithobates pipiens*, and eastern tiger salamander, *Ambystoma tigrinum*. In general, all PFAS compounds reached steady state concentrations within two days of initial exposure. Mean bioconcentration factors were similar among species, but varied among compounds, from 1 (PFOA, PFHxS, and 6:2 FtS) to 100 (PFOS). Larvae exposed to individual PFASs for 30 days (10 to 1000 ppb) exhibited a 3-10 % increase in snout-vent length relative to the controls, but time to metamorphosis was not affected (toads only). The lack of developmental effects was likely a consequence of the duration of our initial assays, which did not encompass metamorphic climax for most of the species tested. We also conducted an experiment to assess the potential influence of PFAS mixtures (PFOS and PFOA) on larval growth in bullfrogs (*Lithobates catesbeianus*). In this longer 72-day study, we found that PFASs generally acted additively to suppress the growth of larvae, suggesting that adverse effects of mixtures might be predictable based on total PFAS concentration. Given the additive nature of some PFASs and our observations of growth effects at 10 ppb, there appears to be potential for adverse outcomes in surface waters where median PFAS concentrations exceed 10 ppb. Further, we observed significant developmental delays associated with the lowest PFOS concentration tested (i.e. 144 ppb), but not PFOA up to 288 ppb. Our results indicate that PFASs have the potential to negatively impact amphibian growth and development at sublethal concentrations. We are currently incorporating semi-realistic mesocosm and field sampling approaches to develop a more comprehensive understanding of the risk posed by PFASs. Together, these studies will allow us to assess how realistic environmental exposures translate into population-level effects for native amphibians.

**369 Comparison Between the Sensitivities of USEPA Endocrine Distruptor Screening Program's Tier 1 and Tier 2 Amphibian Assays Using Select Chemicals**

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The US Environmental Protection Agency, Endocrine Disruptor Screening Program uses a two-tiered approach for screening chemical substances for their potential effect on endocrine systems. The determination whether to require additional Tier 2 testing is made on a weight-of-evidence basis considering data from the Tier 1 assays and other scientifically relevant information that may be available. Three widely used chemicals: 2-ethylhexyl 4-hydroxybenzoate (2-EHHB), 4-nonylphenol-branched (4-NP), and triclosan (TCS), which were selected based on USEPA ToxCast estrogen and androgen receptor in vitro bioactivity data, were subjected to a 21-day Amphibian Metamorphosis Assay (AMA) "Tier 1" and an approximately 16-week Larval Amphibian Growth and Development Assay (LAGDA) "Tier 2". Nieuwkoop and Faber (NF) *Xenopus laevis* larvae at NF stage 51 for AMA and NF stage 8-10 for LAGDA were exposed to 4 test concentrations and a water control. We evaluated the following endpoints during the exposure: mortality, growth (snout-to-vent length (SVL) and body weight), development, morphological features (thyroid, gonad, gonadal ducts, liver and kidney histopathology), overt toxicity, and behavior. We compared the AMA results for TCS from the open literature with the LAGDA results obtained in this study. The AMA studies for the 3 chemicals show no treatment-related effects associated with thyroid-mediated development and no significant histopathologic findings in the thyroid at the tested concentrations. In contrast, LAGDA studies show that the 3 chemicals appear to delay metamorphosis in a concentration-dependent manner, except TCS where the effect is only detected at the highest tested concentration. LAGDA studies also show that there is no effect on growth for TCS and 4-NP, however significant increases in body weight and SVL in NF stage 62 larvae for 2-EHHB were noted. There were no treatment-related findings in the thyroid glands of NF stage 62 frogs exposed to 2-EHHB and 4-NP, however an increase in the prevalence of follicular cell hypertrophy at the minimal severity level was noted in the thyroid glands of frogs exposed to TCS as compared to water controls. It is unclear whether these abnormal morphological features related to exposure to TCS were caused by an endocrine-mediated mechanism. *The findings and conclusions in this abstract have not been formally disseminated by the Agency and should not be construed to represent any Agency determination or policy.*

**370 Altered development of the grass frog (*Rana temporaria*) as a consequence of fungicide induced modifications of food quality**

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Amphibians are threatened worldwide, while stressors of anthropogenic origin such as pesticides seem to be a driving force. Although direct effects are rather frequently assessed, indirect effects of chemicals through an altered nutritious quality of leaf litter for tadpoles have been largely ignored. As the nutritious quality of leaf litter depends on its microbial conditioning, fungicide exposure might induce indirect effects on the larval stage of frogs, such as the grass frog *Rana temporaria*. To assess for such indirect effects, larvae of *R. temporaria* (n=33) were fed over 11 weeks with black alder leaves either leached for 2 days, or conditioned in absence or presence of a fungicide mixture (azoxystrobin, carbendazim, cyprodinil, quinoxyfen and tebuconazole; sum concentration=100 µg/L) for three weeks prior to their introduction into the experiment. Leaves conditioned in presence of the fungicide mixture exhibited a fungal biomass approx. 30% lower relative to the fungicide free control. The consumption of both the leached and the fungicide exposed leaf material caused an elevated feces production (up to 65%) of *R. temporaria*, whereas leaf consumption of the larvae was 20% increased only when leached leaves were offered. These lower food qualities ultimately resulted in a reduced growth (up to 20%). At the same time, metamorphosis was initiated earlier, indicating a potential active avoidance of hostile environmental conditions during the frogs' aquatic life stage. Additional physiological analyses are planned to foster a mechanistic understanding for the observed effects. nonetheless Our data indicate the potential of fungicides to induce indirect effects in frog larvae through the modification of the nutritious quality of leaf litter.

**371 Effects of Road De-Icing Salt (NaCl) and Temperature on Survival and Growth of Larval Anurans (*Lithobates clamitans* & *L. sylvaticus*)**

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An important challenge in amphibian ecotoxicology and conservation is that naturally occurring amphibian populations experience multiple, simultaneous stressors while toxicity tests are usually limited to a single stressor. Amphibian populations in the Mid-Atlantic region, for example, are likely experiencing combined effects of several regionally important stress factors. About 14 million tons of road de-icing salts are used each year in North America with NaCl accounting for 98% of total salt use and, hence, increased salinity is an important environmental stressor to aquatic organisms, including amphibians. As well, higher temperature as a result of climate change is also becoming an increasingly important environmental stressor for amphibians. Unfortunately, there are no data on the combined effects of salinity and temperature on amphibians which can hinder any amphibian conservation efforts in the region. To address this important data gap, we are conducting acute and sub-chronic toxicity tests to explore impacts of these two stressors on two common anurans, the wood frog (*Lithobates sylyaticus*) and the green frog (*L. clamitans*). An acute toxicity test was conducted to determine an NaCl $LC_{50}$ (2587.5 mg $Cl^-$/L), and the results were used to inform the treatment levels of the sub-chronic and chronic tests. In the first sub-chronic NaCl exposure study, we exposed green frog larvae to three temperatures (18, 22, and 25 °C), and three concentrations of sodium chloride (500, 1000, and 2000 mg $Cl^-$/L) for 35 days. The larvae were observed daily for mortality and Gosner stage, as well as length and mass of surviving animals. While there was no significant effect of temperature on mortality ($F_{2,36}$ = 2.984, P = 0.0632), there was a significant effect of chloride ($F_{3,36}$ = 48.029, P = 1.14x$10^{-12}$), and no significant interaction effect was observed ($F_{6,36}$ = 0.954, P = 0.4670). Mortality of individuals in the higher mg $Cl^-$ /L treatment group was significantly higher than that of controls and other treatment groups (2000 mg/L > 1000 mg/L = 500 mg/L = 0 mg/L). The experiments with larval wood frogs are currently ongoing but show similar levels of sensitivity to NaCl compared to the experiments on green frog larvae. These studies provide important insights into effects of multiple environmentally relevant anthropogenic stressors on commonly occurring Maryland amphibians and will likely point to NaCl thresholds relevant to environmental management.

**372 Determination of mechanisms of toxicity of legacy brine contamination to amphibians**

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The Williston Basin, North Dakota, has been a leading producer of oil and gas for decades. Brine, water co-produced during the production of oil and gas, has contaminated both the surface and ground water in the area through historical disposal practices and accidental releases. Much of the Williston Basin is included in the Prairie Pothole region, which is pocketed with wetlands that are home to many amphibians. These amphibians play a crucial role in wetland ecosystems by cycling nutrients between the aquatic and terrestrial environment and serving as both predators and prey in the wetland food web. During the aquatic portion of their life cycles, many amphibians in Williston Basin wetlands are potentially exposed to brine contamination, typically marked by high concentrations of total dissolved solids, sodium, and chloride, yet little is known about the toxicity of brine to amphibians. To determine if the ion mixture associated with brine contamination was responsible for mortality observed in previous work, we exposed boreal chorus frog tadpoles (*Pseudacris maculata*) to 1 of 4 reconstituted water treatments for two weeks. The water was mixed to mimic water from a reference wetland and a brine contaminated wetland. Additional treatments included the brine contaminated wetland water spiked with NaCl to 1000 or 2000 mg/L Cl to understand whether chloride was driving the response. Endpoints evaluated included mortality and behavior of the tadpoles as well as measures of biochemical stress. These data clarify the relative importance of chloride in driving toxic effects of brine to help with risk management of waters from oil and gas operations and inform future restorations of amphibian habitat.

**373 Exploring the amphibian exposome in an agricultural landscape using telemetry and passive sampling**

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Pesticides are one of multiple stressors that contribute to amphibian declines, but their population-level effects are still poorly understood. To determine effects, we must have a better understanding of the route, timing, and rate of exposure under field conditions for various amphibian species. The exposome is defined as a measure of an individuals exposure to all stressors from birth to death and how those exposures relate to health. Understanding the complex relationships among amphibians, pesticides, and their environments is vital for conservation, and investigations into pesticide exposure in the field is a central piece of this puzzle. Specifically, to design mitigation strategies, we need to know where (habitat type) and when (seasonally) individuals are at the highest risk of pesticide exposure. We designed the first field study of its kind that combined radio telemetry, passive samplers, and pesticide accumulation in tissues to characterize the amphibian exposome as it relates to pesticides. Understanding how habitat drives exposure in individuals (i.e., their exposome), and how that relates to individual health is critical to managing species in an agricultural landscape where pesticide exposure is likely. We radio-tracked 72 northern leopard frogs (*Lithobates pipiens*) in two agricultural wetlands to understand where and when individuals are at the greatest risk of pesticide exposure. Novel silicone passive sampling devices (PSDs) were deployed at sites where telemetered frogs were located, then moved to subsequent locations as frogs were radio-tracked. Pesticide concentrations in PSDs varied by habitat and was greatest in agricultural fields where frogs were rarely found. Pesticide concentrations in frogs were greatest in spring when frogs were occupying wetlands compared to late summer when frogs occupied terrestrial habitats. Our results indicate that habitat and time of year influence exposure and accumulation of pesticides in amphibians. Our study illustrates the feasibility of quantifying the amphibian exposome to interpret the role of habitat use in pesticide accumulation in frogs to more effectively conserve amphibians in agricultural landscapes.

## Advancing the Adverse Outcome Pathway Concept for Mitochondrial Dysfunction

### 374 Mitochondrial responses for exploring heterogeneous resolutions of contaminant bioavailability using cell-based in vitro assays

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With the intensive developments induced by rapid industrialization and extensive urbanization, increasing amounts of anthropogenic chemical contaminants have been continuously discharged into aquatic environments. While using traditional methods, which are mostly based on the chemical analysis alone of extracted samples from environmental and biological matrixes, it is difficult to accurately determine the bioavailability of contaminants and then reflect their cell-based effects as well as subsequent toxicity risks. Therefore in this study, understanding the degrees of contamination and pollution characteristics regarding of entirely environmental matrix and biological samples in the selected study sites and successfully culturing the primary cells from critical tissues in aquatic organisms of ecological niche, the field exposure were performed to analyze the subcellular distributions of contaminants and adverse effects of chemicals combined with cell-based in vitro assays and in situ tests. Further, the dynamic exchanges, translocation and mobilization of contaminants such as metals were then clarified among the "various particles, pore water and cells in exposed organisms" interfaces of matrix through measuring relevant dynamic parameters and resultant induced fluxes during experimental procedures. Moreover, coupled with chemical analysis to quantify the mitochondrial components (mitochondrial proteins, lipids, and nucleic acids, modulating mitochondrial membrane potential and ATP production et al.,) within culture wells of primary cells and molecular approaches to assess the expression of responsive genes in field exposure, the mechanisms of associated toxicity were elaborated and the interactive relationships were then obtained between chemical bioavailability and in-situ stress. Simultaneously, the assays of cell-based evaluation and monitoring were established considering the consistency of framework as protocols among cell culture, field tests and exposure effects. Overall, the adverse outcomes acquired in this study support full-scale considerations of environmental prevention and retrieval and thus provide robust tool to scientifically extrapolate the environmental quality guidelines (EQGs), further improving accuracy and ecological relevance towards future pollution monitoring as powerful tools and more comprehensive risk assessments in complex scenarios. (The author acknowledges financial support by Grant No. 21777156/B070403 from National Nature Science Foundation of China).

### 375 Identifying genomic networks associated with mitochondrial dysfunction: A meta-analysis approach

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Environmental chemicals can affect mitochondrial function and lead to adverse effects on survival, growth, reproduction, and development in wildlife. Adverse outcome pathways (AOPs) have provided a framework to link mitochondrial dysfunction to apical responses, but further work is needed to establish causality through intermediate key events. Characterizing these key responses are important for AOP construction and required to provide meaningful biomarkers for mitochondrial dysfunction. Thus, the objective of this study was to identify transcriptome networks associated with mitochondrial dysfunction. Briefly, rank order gene lists (ROGLs) were generated for all chemicals in the Comparative Toxicogenomics Database consisting of 5 or more gene interactions for "mRNA:increases^expression" and "mRNA:dereases^expression". We then leveraged high-throughput screening data from the Tox21 program to identify chemicals that decreased the mitochondrial membrane potential. Specifically, chemical records were classified based on hit calls (i.e., active or inactive) in the "TOX21_MMP_ratio_down" assay. Finally, the percent rank of each gene (based on the ROGLs) was compared between active and inactive substances for "mRNA:increases^expression" (11525 genes) and "mRNA:dereases^expression" (10977 genes). It was found that 237 genes were significantly enriched among active compounds for increased mRNA expression, and 152 genes were enriched for decreased mRNA expression ($p < 0.05$). Gene lists were then leveraged in an over-representation enrichment analysis in WebGestalt to identify gene ontologies and functional pathways associated with mitochondrial dysfunction. It was found that biological processes related to lipid metabolism and carboxylic acid metabolism were upregulated, while steroid metabolic processes and responses to hormones were downregulated. Similar results were obtained in a functional pathway analysis; significant Reactome pathways for upregulated transcripts associated with mitochondrial dysfunction were related to lipid metabolism (e.g., beta-oxidation of mitochondrial fatty acid) as well as nuclear receptor regulation and estrogen-dependent gene expression. Thus, the results of this analysis indicate that altered lipid metabolism and endocrine signaling are common responses associated with mitochondrial dysfunction, which may lead to subsequent adverse effects in defined AOPs.

### 376 High-throughput chemical screening of mitochondrial toxicants using zebrafish embryos

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Mitochondrial dysfunction is a prevalent molecular event that can result in multiple adverse outcomes. Recently, a novel high throughput method to assess metabolic capacity in fish embryos following exposure to chemicals has been adapted for environmental toxicology. Assessments of oxygen consumption rates using the Seahorse XF(e) 24/96 Extracellular Flux Analyzer (Agilent Technologies) can be used to garner insight into toxicant effects at early stages of development. Here we synthesize the current state of the science using high throughput metabolic profiling in zebrafish embryos, and present considerations for those wishing to adopt high throughput methods for mitochondrial bioenergetics into their research. Chemicals that have been investigated in zebrafish using this metabolic platform include herbicides (e.g. paraquat, diquat), industrial compounds (e.g. benzo-[a]-pyrene, tributyltin), natural products (e.g. quercetin), and anti-bacterial chemicals (i.e. triclosan). Some of these chemicals inhibit mitochondrial endpoints in the µM-mM range, and reduce basal respiration, maximum respiration, and spare capacity. We present a theoretical framework for how one can use mitochondrial performance data in zebrafish to categorize chemicals of concern and prioritize mitochondrial toxicants. Noteworthy is that our studies demonstrate that there can be considerable variation in basal respiration of untreated zebrafish embryos due to clutch-specific effects as well as individual variability, and basal oxygen consumption rates (OCR) can vary on average between 100-300 pmol/min/embryo. We also compare OCR between chorionated and dechorionated embryos, as both models are employed to test chemicals. After 24 hours, dechorionated embryos remain responsive to mitochondrial toxicants, although they show a blunted response to the uncoupling agent carbonylcyanide-4-trifluoromethoxyphenylhydrazone (FCCP); dechorionated embryos are therefore a viable option for investigations into mitochondrial bioenergetics.

We present an adverse outcome pathway framework that incorporates endpoints related to mitochondrial bioenergetics. High throughput bioenergetics assays conducted using whole embryos are expected to support adverse outcome pathways for mitochondrial dysfunction.

**377 Oxidative Stress and Apoptosis in Zebrafish (*Danio rerio*) After Exposure to Fungicides at Environmentally Relevant Concentrations**

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Fungicides are widely used to treat fungal disease in agricultural crops, animals, and humans. Recent studies have shown a significant amount of fungicides in streams, groundwater, and sediments. In the current study, we compared the non-target toxicity of two frequently applied classes of fungicides (azole and strobilurin) using larval zebrafish (*Danio rerio*). The goal of this project was to investigate relative fungicide toxicity using behavioral endpoints and molecular biomarkers associated with their mode of action. Zebrafish embryos were exposed to fungicides (tebuconazole, propiconazole, myclobutanil, azoxystrobin, and pyraclostrobin) at different environmentally relevant concentrations, 10 ng/L, 100 ng/L, 10 ug/L, 100 ug/L and 1 mg/L, starting at 4 h post- fertilization (hpf) to 5 days post fertilization (dpf). Behavior assays were conducted to examine swimming behavior (total distance and velocity) at 5 dpf using Danio Vision®. The stability of fungicides under experimental conditions was conducted and analyzed using LC-MS/MS. mRNA expressions (CYP51, GST, Casp9, p53, and BAX), and enzymatic activities (ROS, Casp3, TBARS) were measured to assess the relative toxicity amongst fungicides. The velocity and distance traveled by zebrafish larvae when exposed to azole fungicides (tebuconazole, propiconazole, and myclobutanil) were not significantly different from the controls. However, in the case of the strobilurin chemical class, there was a significant decrease in the swimming behavior of the fish larvae. The molecular biomarkers indicate that strobilurin fungicides and propiconazole may cause oxidative stress and apoptosis at environmentally relevant concentrations. The results for the mitochondrial dysfunction are forthcoming.

**378 Mitochondrial Dysfunction Contributes to the Persistent Effects of Benzo(a)pyrene Across Multiple Generations: Mechanisms and Consequences**

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The potential for polycyclic aromatic hydrocarbons (PAHs) to have effects across generations is an emerging concern in environmental health; however, the underlying mechanisms are poorly understood. In the present study, we explore role of mitochondria in the multigenerational toxicity of PAHs. Specifically, we characterize the persistent bioenergetic effects of benzo(a)pyrene (BaP) – a model PAH and known mitochondrial toxicant – in F1 (maternally exposed), F2 (cross-generationally or germline exposed), and F3 (transgenerationally exposed) generations following a chronic maternal (F0) dietary exposure using the model teleost *Danio rerio*. Maternally exposed F1 embryos exhibit reduced mitochondrial DNA integrity, reduced mitochondrial function and efficiency, and impaired antioxidant defense systems during development, largely in the absence of effects in exposed F0 females. Metabolic shifts during development create potential for disease pathologies and reduced organismal fitness later in life. In F1 adults, mitochondrial dysfunction presents in cardiac tissue with reductions in mitochondrial reserve capacity. Maternally BaP exposed F1 fish also exhibit altered behavioral, metabolic, and reproductive phenotypes. PAH-induced changes in mitochondrial function and metabolic plasticity persist in the F2 embryos, manifesting at F0 exposure levels that do not cause significant dysfunction in the F1 generation, with important implications for the persistent effects of pollution in the environment. The ability to adjust metabolism is crucial for organisms to effectively respond to a variety of natural and anthropogenic stressors, suggesting that organisms with alterations in fundamental bioenergetic processes may be more sensitive to secondary stressors. Herein we demonstrate that F2 fish exhibit altered metabolic response to thermal stress, reduced thermal tolerance, and fitness tradeoffs. Cross-generational exposure to BaP potentiates metabolic effects under thermal stress even in the absence of effects at baseline temperature. Current studies are evaluating whether mitochondrial dysfunction persists in transgenerationally exposed F3 fish. Taken together, these data suggest that mitochondrial dysfunction contributes to the multigenerational toxicity of BaP, with important implications for human disease pathologies and organismal ecological fitness.

**379 Long-term effects of early-life mitochondrial toxicity in the context of genetic deficiencies**

*J.N. Meyer, D.F. Mello, C. Bergemann, Duke University / Nicholas School of the Environment; K. Hershberger, I. Ryde, Duke University; A. Luz, NIEHS; T. Leuthner, C. Gonzalez-Hunt, Duke University*

Many environmental pollutants target mitochondria, but the mechanisms by which they act vary, including mitochondrial DNA damage (e.g., benzo[a]pyrene), protein inhibition (e.g., arsenic, rotenone), mitochondrial uncoupling (e.g., pentachlorophenol), and redox cycling (e.g., paraquat). Many chemicals have multiple molecular targets. Furthermore, growing evidence suggests that the toxic effects of certain chemicals on mitochondrial function can be highly persistent. This may be especially true of developmental exposures, since mitochondria undergo biogenesis and major functional changes during early development and cellular differentiation. We found that exposure of first larval stage *Caenorhabditis elegans* to arsenic as well as ultraviolet C radiation (UVC; employed to caused persistent mitochondrial DNA damage) exposure caused lifelong decreases in ATP, while rotenone caused lifelong increases. These exposures also led to significant remodeling of mitochondrial energy metabolism: arsenic caused disruption of pyruvate metabolism and Krebs cycle activity, UVC caused changed in insulin signaling and mitochondrial oxidative stress pathways, while rotenone resulted in activation of the glyoxylate and glycolytic pathways in an apparently compensatory fashion. Mutations in mitochondrial fusion, fission and autophagy genes exacerbate the toxic effects of other environmental agents including arsenic, rotenone, and paraquat, underscoring the importance of these homeostatic pathways in defending against mitochondrial toxicants. We are currently examining the effects of additional mitochondrial toxicants, including pentachlorophenol and pyraclostrobin. We are also testing whether mitotoxicants acting on different molecular targets have common effects on dopaminergic degeneration and activity levels, hypothesized to be common downstream outcomes of mitochondrial dysfunction.

**380 Rotenone-induced mitochondrial dysfunction alters pathogen resistance in Caenorhabditis elegans**

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Growing evidence have shown that mitochondrial dysfunction not only compromises the energetic metabolism of cells, but also play key roles in other physiological processes such as immunomodulation. We hypothesize that mitochondrial toxicity can be a common link between increased prevalence in immune-related disorders and toxic environmental exposures. To test this hypothesis, we are using the pesticide rotenone –a widely known mitotoxicant that inhibits complex I– and the model organism *Caenorhabditis elegans*. Worms were dosed twice in liquid since eggs at 0 and 24h to 0.25 and 1 uM Rotenone (Rot) or vehicle (1% DMSO; Ctrl) and harvested at 48h. After a further 48h of detoxification period, worms were used to analyze: body length; oxygen consumption rates (OCR); nuclear (nDNA) and mitochondrial (mtDNA) DNA copy number; and survival rate in *Escherichia coli* strain OP50, *Pseudomonas aeruginosa* strain PA14, and *Salmonella enterica* serovar Typhimurium strain SL1344. Our preliminary results show that Rot-exposed worms presented a dose-dependent decrease in body length, and a decrease in basal, ATP-linked and non-mitochondrial OCR, as well as mitochondrial spare capacity. The mtDNA:nDNA ratio showed a trend to higher levels only in worms treated with Rot 1uM. Interestingly, Rot-exposed worms lived longer than Ctrl animals (13% increase in median survival for both doses), but were more susceptible to PA14 (12% decrease in median survival for both doses). However, worms treated with the highest Rot dose revealed a higher resistance to SL1344 (11% increase in median survival), whereas worms exposed to the lower dose were as susceptible as the Ctrl group. These same analyses were also conducted with the offspring (F1) of the Rot exposed worms (P0), however our preliminary results do not support any heritable effects for the measured phenotypes. Altogether our findings suggest that early life exposure to rotenone can promote later life mitochondrial dysfunction (evidenced by the decreased mitochondrial respiration parameters) and alter the capacity of individuals to fight pathogenic bacteria. Next, we will perform transcriptomic and epigenetic analysis to further understand the mechanisms behind the cross-talk between mitochondrial function and immunocompetence.

**381 Twinkle twinkle little StAR how did things go this far: A case study on the development of an incorrect AOP and the correct AOP**

*S.L. Levine, Monsanto Company / Global Regulatory Sciences*

Mitochondrial effects are frequently over looked as a mechanism of action for an observed effect either in in vitro or in vivo test systems. Often an observed effect *in* in vitro or in vivo studies assays can be related to a nonspecific cytotoxic effect on mitochondrial structure and function. This presentation will provide an informative case study where an adverse outcome pathway (AOP) was incorrectly developed for a specific effect on steroidogenesis rather than for a nonspecific effect to steroidogenesis through an effect on mitochondrial structure and function by a surface-active chemical at supraphysiological concentrations. The original study incorrectly concluded that the surface-active chemical had a direct effect on expression of the Steroidogenic Acute Regulatory (StAR) protein, which is widely considered to be the initial and rate limiting step in steroidogenesis. This presentation will highlight the oversight that was made in the research that developed the original AOP, which subsequently became well-accepted in the literature, and how a rigorous secondary evaluation of mitochondrial structure and function was used to characterize the actual mechanism of action. In addition, the results from this analysis have been placed into an AOP, with disruption the mitochondrial electrochemical gradient as the molecular initiating event, and has been put this effect into the context of an environmental risk assessment.

## Modeling and Data Analysis Tools to Predict the Fate and Transport of Pesticides in the Environment

**382 An integrated RICEWQ-AnnAGNPS modeling system to evaluate the fate and transport of Thiobencarb in Colusa Basin, Northern California**

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As a pre-emergent or early post-emergent herbicide, thiobencarb is widely used in Northern California rice fields. Released paddy water containing thiobencarb may pose ecological risks to non-targeted organisms living in downstream water bodies. Current watershed models usually lack proper mechanisms to accurately represent the complicated hydrological and water quality conditions in rice fields. Therefore, they are not suitable to capture the fate and transport of thiobencarb in rice fields. In this research, we proposed an integrated modeling system: RICEWQ-AnnAGNPS to correctly reflect the dynamics of thiobencarb at rice fields, as well as the subsequent processes after paddy water is discharged and transported to downstreams. RICEWQ model, which is specifically designed for rice fields, is first setup at all rice paddies in Colusa Basin to simulate paddy water discharge and thiobencarb exposure at the edge of fields. Watershed model, AnnAGNPS is setup in the same region to capture the flow discharge from landuses other than rice. Landscape processes of thiobencarb from fields to reaches and channel routings are also simulated by AnnAGNPS. Consequently, the impact of upstream rice pesticide application on exposures at receiving water bodies can be evaluated, with the implementation of the proposed coupled modeling system in Colusa Basin, Northern California.

**383 Methodology for Prioritizing Pesticides for Surface Water Monitoring in Agricultural and Urban Areas of California**

*Y. Luo, California Department of Pesticide Regulation; X. Deng, California EPA / Surface Water Protection Program; R. Budd, Cal EPA / Dept. of Pesticide Regulation; M. Ensminger, CDPR / Environmental Monitoring*

California Department of Pesticide Regulation (CDPR)'s Surface Water Protection Program is developing a more consistent and transparent modeling approach for the prioritization of pesticides for surface water monitoring in California. A computer model, Surface Water Monitoring Prioritization (SWMP) model, has been developed (http://cdpr.ca.gov/docs/emon/surfwtr/sw_models.htm) with CDPR's Pesticide Use Reporting (PUR) database as the primary input data. Data from USEPA Aquatic Life Benchmarks, IUPAC Pesticide Property Database, and USGS National Hydrography Dataset are also incorporated into the model. The model generates monitoring priority lists for pesticide ingredients and their degradates, and optimizes monitoring project planning by answering the questions: where, when, and what to sample. For consistency with the approach used in agricultural areas, urban PUR data are converted from county scale to watershed scale by considering population density and watershed delineation. This represents one of the first systematic and practical applications of PUR downscaling. Refined analysis on pesticide use data is also incorporated in the recent model improvement for the determination of Areas of Interest (AOI's) and associated Pesticides of Interest (POI's) for monitoring. The model output now provides a short list of HUC12's watersheds (out of the 4,415 HUC12 watersheds in California) and candidate streams to be considered for site selection in a monitoring project.

**384 Time series analysis of pesticide air concentrations--effects of mitigations in selected California agricultural communities**

*F. Sartori, CA Dept of Pesticide Regulation*

The California Department of Pesticide Regulation (CDPR) created the Air Monitoring Network (AMN) to monitor ambient air concentrations of soil fumigants and other pesticides in the three agricultural communities of Ripon, Salinas, and Shafter, California. Time series analysis of AMN air concentrations (2011–2016) for the soil fumigants, methyl bromide (MeBr), methyl isothiocyanate (MITC), and 1,3-dichloropropene (13D), was conducted to assess the effects of federal and state mitigations to reduce pesticide air concentrations. Mitigations directed at reducing pesticide use were broadly classified as use-related factors (UF), whereas those at emission reduction as emission-related factors (EF). The model development methods for intervention analysis also known as Box-Tiao modeling were implemented using SAS PROC ARIMA. Input variables included the corresponding time series of pesticide use, an *ad-hoc* indicator, "season", for the months of peak air concentrations, and intervention indicator variables. "Intervention" was any abrupt change in a time series' mean, variance, or both that was also statistically significant based on the selected ARIMA models. The timing of interventions corresponding to a specific mitigation was either known or unknown. Analyses were performed twice: with non-detects replaced by a constant value of half the laboratory limit of detection (LOD), and by simulated data via maximum likelihood estimation based on the distribution of the values above the LOD. The significance of the use parameter, as well as the autoregressive parameter at lag 12 months, indicated that detections were related to use in a cyclical—mostly annual—pattern for all three fumigants in most locations. The MeBr air concentrations were decreasing overtime with interventions occurring at the end of 2015 in all three locations, supporting evidence that the timing of these interventions coincided with that of both the USEPA UF mitigations and 2015 CDPR EF mitigation. In general, there was a small but significant decrease in MITC concentrations in 2015 for Ripon and Salinas, supporting evidence that both the USEPA and CDPR EF mitigations were effective. For the 13D time series, there were no significant interventions that may be associated with any EF- or UF-type mitigation based on the selected model; and the overall trend remained somewhat constant, likely due a constant 2011–2016 demand in its use to compensate for the decline in MeBr use.

**385 Sorption of phenylurea herbicides by soils: Modelling uncertainty and ruling factors with an artificial neural-based fuzzy inference system**

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Retention of pesticides by soils is both spatially variable and also one of the most sensitive factors determining losses to surface and groundwater. To date, only a few work has been done to explain this process in tropical soils especially, and generally to uncover the factors that govern the process in both temperate and tropical soils. The purpose of this study was therefore to evaluate the influence of various and interrelated specific soil properties and the pesticide specific molecular descriptors on sorption of pesticides in tropical soils, and to develop and test a simple explainable sorption model. The sorption behavior of five representatives of the phenylurea family (PUH) were studied in twelve soils of contrasting characteristics, stemming from the Southwestern Nigeria. Sorption isotherms and coefficients were obtained by equilibrating the soil samples with 0.01M $CaCl_2$ solutions spiked with increasing concentrations of the target PUHs. HPLC-DAD was used to quantify the target PUHs in solutions at equilibrium. The isotherm data were fitted to Freundlich and linear distribution equations ($R^2 \geq 0.96$ and $R^2 \geq 0.76$, respectively) to obtain the isotherm and distribution coefficients ($K_f$, and $K_d$). Spearman rank correlation was used to determine the specific soil and PUHs properties that have significantly high correlations with $K_f$ or $K_d$. Significant correlations were established between $K_d$ or $K_f$ and the following factors: soil properties (pH, cation exchange capacity, organic carbon, extractable Fe and Mn content, and mass proportions of clay and silt), and the pesticide molecular descriptors [molecular weight ($M_w$) and hydrophobicity index ($logK_{ow}$)]. These were used alongside with PUHs concentrations as potential descriptors. However, due to the problem of multicollinearity between these predictors, multiple linear regression and other similar statistical approach were inadequate to elaborate a model, hence the use of an artificial neural-based fuzzy inference system (ANFIS). Several models of different combinations of the factors were then elaborated under 10-fold cross-validation. Furthermore we carried out a sensitivity analysis of the factors to establish the most important predictor. In conclusion, we successfully used ANFIS to construct a holistic 4 factor system that could model the sorption of PUHs in tropical soils. The model should assist in understanding the fate of pesticides, and for their risk assessment in tropical soils.

**386 Evaluation of Climate Change Effects on Natural Sediment Recovery in an Alpine Lake**

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Assessing the role of climate change on sediment and contaminant fate and transport is of increasing importance in remedial investigations to accurately evaluate time scales over which recovery can be expected in natural environments. To this effect, the authors undertook a detailed modeling study, validated by field measurements, which examined the role of climate change on sediment fate and transport in an alpine lake in Northern Italy (Lago Maggiore, Italy). The transport of sediment and sediment-bound contaminants throughout Lago Maggiore is important to evaluating and managing long-term environmental risks. As a part of the investigation of sediment dynamics in Pallanza Bay (an embayment of Lago Maggiore, fed by the Toce River), a modeling study was conducted to better understand hydrodynamic processes and the stability of sediment and contaminants, DDT in particular. Sediment dynamics in Lago Maggiore were investigated under two climate change scenarios, as delivered by the Intergovernmental Panel on Climate Change. The two climate models EC-EARTH and ECHAM6 under the Representative Concentration Pathways (RCPs) 8.5, were applied to a previously calibrated and validated hydrodynamic and sediment transport model of Lago Maggiore. These scenarios provided the maximum and minimum river flow and sediment load conditions to the lake, respectively. The predicted deposited sediment was characterized in terms of cumulative thickness, annual average deposition rate, and spatio-temporal distribution trends over a 34 year period (2016-2050). Results of the climate change scenarios indicate that the EC-EARTH RCP 8.5 scenario (with higher flow rates and sediment loading) resulted in higher sediment deposition outside of the river mouth and transported sediment further into Pallanza Bay, similar to the deposition pattern measured by geochronology cores. The ECHAM6 RCP 8.5 scenario with lower flow rates and sediment loading predicted lower sediment deposition near the river mouth and less sediment transport into Pallanza Bay. The predicted sediment deposition rates for ECHAM6 throughout Pallanza Bay were in good agreement with the model hindcast data and historical measurements. The proposed climate change scenarios provide an assessment of potential future deposition patterns that are reasonable under the expected evolution of climate change within the next half century.

**387 The Pesticide Emission Model (PEM): A tool for assessing pesticide volatilization from agricultural applications**

*S. Ghosh, K. Crist, Ohio University / Chemical & Biomolecular Engineering; S. Grant, Syngenta Crop Protection LLC*

Predicting the atmospheric fate and transport of pesticides is complex and challenging due to the compounding factors of the localized environment (e.g. environmental conditions, crop, topography) and physical properties of the pesticide. The current work introduces the development of the Pesticide Emission Model (PEM), a deterministic model that predicts diurnal volatilization emission flux rates after agricultural spray applications to soil and plant surfaces. The PEM utilizes dynamic transport processes, fate mechanisms, physicochemical properties and environmental conditions (e.g. temperature, wind speed, solar radiation, precipitation, land cover, leaf area index (LAI), etc.) to determine mass transfer rates from the sprayed surface to the atmosphere. Validation of the PEM model shows strong agreement with hourly observations of soil and plant volatilization. A user interface was also developed to position PEM in a more user-friendly platform. This interface couples the modeled emission/volatilization rates with AERSCREEN and AERMOD dispersion models to assess off-target deposition and air concentrations; thereby, informing risk assessments by providing greater insight about potential off-target exposure of pesticides.

**388 AGDISP: Canopy Interactions and Future Development**

*H. Thistle, USDA Forest Service / FHAAST Forest Health Protection*

The AGDISP computer model is used to estimate pesticide deposition from various application scenarios. The model code is dynamic and the research version undergoes changes regularly. The nature of this model and other deposition models will be discussed as well as future model development directions. Recently, two forest deposition data sets became available to test against AGDISP predictions. Both scenarios require physics that either are not in AGDISP or are treated in an extremely simplistic manner. One data set is of in-canopy deposition in a deep coniferous canopy and one data set shows deposition in rowed trees in a young conifer plantation (this geometry violates the continuous canopy assumption in AGDISP). The experimental data is discussed, how these types of data sets are used to improve AGDISP is described and potential approaches to improve the model physics are presented.

**389 Towards Improved Modeling of Pesticide Volatilization: Using Measured Soil-Air Partition Coefficients in a Pesticide Volatilization Model**

*S. Das, University of Otago / Chemistry; K.J. Hageman, Utah State University / Department of Chemistry and Biochemistry*

Volatilization can cause significant loss of pesticides from agricultural fields, leading to reduced efficacy of the pesticide and harm to non-target ecosystems. Our research group previously developed a model to predict the cumulative volatilization losses of pesticides from agricultural fields, based on environmentally relevant partition coefficients and the mass-balance distribution of pesticides between soil, air, and water compartments. Originally, we used $K_{soil\text{-}air}$ partition coefficients for pure active ingredients input values in the model. However, to better understand and predict pesticide volatilization, we need to know how partition coefficients are affected by the chemicals (e.g. solvents, wetting agents, solvents, dyes and/or other adjuvants) found in real commercial pesticide formulations. In addition, surfactants designed to decrease pesticide volatilization are sometimes added separately to spray tanks, but little actual data about their effects on pesticide volatilization exist in the literature. In this project, a modified version of a fugacity meter was used to measure the $K_{soil\text{-}air}$ values of three semi-volatile pesticides (trifluralin, chlorpyrifos & pyrimethanil) (a) as pure active ingredients, (b) in a typical commercial formulation, and (c) in the presence of active ingredient or commercial formulation with additional surfactant, Synoil™, which is sold to enhance pesticide uptake and spreading. Experiments were conducted at several environmentally relevant temperatures (10-30 °C). We found that the $K_{soil\text{-}air}$ values in commercial formulations (i.e. $K_{soil\text{-}air,\ formulation}$) were more than 10 times lower than those for pure active ingredients. For example, the log $K_{soil\text{-}air}$ for pure pyrimethanil was 8.44 whereas that for pyrimethanil in its commercial formulation was 7.28. However, commercial surfactant Synoil™ had minimal effect on $K_{soil\text{-}air}$ values. When the $K_{soil\text{-}air,\ formulation}$ values were used as input values in the pesticides volatilization model, the cumulative percentage volatilization for 24 hours increased by up to five times compared to when using the $K_{soil\text{-}air}$ value for the active ingredient alone. This suggests that a better qualitative understanding of how adjuvants and surfactants affect pesticide-soil interaction is needed.

## Fate and Effects of Metals – Regulatory and Risk Assessment Perspective

**390 Multiple Linear Regression Models for Developing Bioavailability-adjusted Freshwater Criteria for Lead**

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Metals bioavailability and toxicity may be predicted using mechanistic approaches, such as the biotic ligand model (BLM), and empirical approaches, such as multiple linear regression (MLR). BLMs for lead (Pb) in freshwater have been developed and used to propose bioavailability-based ambient water quality criteria and threshold effect concentrations in the United States and Europe. For regulatory applications in the United States, interest is developing in using empirical approaches that consider the influence of multiple water chemistry parameters on metals bioavailability (such as dissolved organic carbon [DOC], pH, and hardness). The U.S. Environmental Protection Agency (USEPA), for example, recently recommended draft MLR-based aquatic life criteria for aluminum. In the present evaluation, we developed MLR-based models for Pb and three forms of aquatic life (algae, invertebrates, and fish). In general, Pb MLR models were able to predict toxicity with a level of accuracy that is comparable to the BLM. The Pb MLR models were then used to derive potential acute and chronic MLR-based Pb criteria following current USEPA guidelines for criteria development, as well as using alternative approaches (such as inclusion of algae in the genus sensitivity distribution). Lead MLR models and BLMs, and potential criteria using each, will be compared and assessed.

**391 Development of the Draft Canadian Water Quality Guidelines and Ecological Screening Assessment for Manganese Using a New Statistical Approach**

*R.L. Dalton; K. McTavish, T. El-Fityani, Environment and Climate Change Canada; D.J. Spry, Environment and Climate Change Canada / Water Policy & Coordination Direct*

Manganese (Mn) is a naturally-occurring and abundant metal, as well as an essential trace element. Globally, manganese is the fourth most widely used metal after iron, aluminum and copper. Manganese is widely used as an additive and element in the production of steel and other alloys, as a component of dry-cell alkaline batteries, and in a wide range of other products. Manganese enters the environment from geological weathering, as well as from point sources from a variety of industrial sectors including: pulp and paper, wastewater and sewage treatment, metal ore and coal mining, and bitumen extraction. As part of the current phase of the Chemicals Management Plan (CMP), Environment and Climate Change Canada and Health Canada are assessing the potential for manganese to cause harm to the environment and to human health under the Canadian Environmental Protection Act, 1999. Concurrently, draft Canadian Water Quality Guidelines (CWQG) for the protection of freshwater aquatic life for manganese are being developed through the Canadian Council of Ministers of the Environment (CCME). The draft long-term guideline values incorporate the bioavailability and toxicity modifying factors pH and hardness. The draft CWQG for manganese incorporates a new statistical method that fits species sensitivity distributions using maximum likelihood estimation coupled with a model averaging approach. For a given pH and hardness, the 5th percentile of the model weighted-average species sensitivity distribution becomes the long-term CWQG and the predicted no effect concentration (PNEC). The advantages of this new approach will be discussed, as well as how the approach will be used in the CMP draft ecological screening assessment for manganese and its compounds.

**392 Update on the 2018 Aluminum Ambient Water Aquatic Life Criteria**

*D.M. Eignor, K. Gallagher, USEPA / Office of Water*

The US Environmental Protection Agency (EPA) is updating its 1988 recommended aquatic life criteria for aluminum under Clean Water Act Section 304(a). The 1988 criteria account for the influence of pH on toxicity, similarity of acute toxicity of fish and invertebrates, and the greater sensitivity of invertebrates in chronic toxicity tests. The 1988 document recommended an acute criterion of 750 µg/L and a chronic criterion of 87 µg/L when the pH is between 6.5 and 9.0. The effects of hardness and dissolved organic carbon (DOC) were not considered in the 1988 criteria derivation. Over the past 25 years, new acute and chronic toxicity data for both freshwater and saltwater organisms have been published. Additional toxicity data for the eight recommended families as well as mussel toxicity studies are included in the new evaluation. EPA compared several approaches that reflect water quality condition impacts on toxicity including complete and simplified aluminum Biotic Ligand Models (BLMs) and multi-linear regression equations using pH, hardness, and DOC parameters. Comparisons were conducted to facilitate evaluation of the various approaches for criteria development. The multi-linear regression approach using pH, hardness, and DOC parameters was selected for criteria development based on the work of DeForest *et al.* 2018. This approach was selected due to 1) the transparency of the model, 2) the similarity and accuracy of the results relative to the available BLM model outputs and empirical data, and 3) the decreased number of input water chemistry data needed to derive criteria at different sites, which increases utility of the model for end-users.

**393 Models, beakers, and ecosystems: Utilizing linked biotic ligand and biodynamic models to predict accumulation in field collected invertebrates.**

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Models predictive of metal accumulation and effects generally fall into two types of models, biodynamic- and biotic ligand-style models. Each comes with advantages and disadvantages. Biodynamic models account for species-specific process that control metal accumulation under simplified lab scenarios but have limited utility to ecosystem management because they are difficult to extrapolate to food webs and rarely predict effects on organisms. Biotic ligand models predict the lethality of metals due to differences in water quality on metal speciation. These models are more easily extrapolated to ecosystems but they do not account for the biological processes known to control metal accumulation. To improve the prediction of metal accumulation and effects to ecosystems we developed a linked biotic ligand-biodynamic modeling framework. To do so we first developed a framework using mesocosm data of single and metal mixture accumulations into periphyton and the aquatic insect *Brachycentrus americanus.* We then used this model to predict metal accumulation into field mayflies and caddisflies. Model predictions suggest while more than 80 percent of the metal accumulated into insects (mesocosm or field) was acquired via the diet; bioavailability of metals to periphyton was described by solution chemistry. The metals accumulation was in turn predictive of adverse effects to communities. We utilized advantageous aspects of both modeling paradigms and thus made strides at developing a model of metal accumulation into food webs that could be applied to the management of ecosystems.

**394 Long-term effects of metal-contaminated sediments from streams in the Tri-State Mining District on juvenile freshwater mussels**

*J.M. Besser, J.L. Kunz, C.D. Ivey, D. Cleveland, J.A. Steevens, USGS / Columbia Environmental Research Center*

Field studies have documented that the abundance and diversity of freshwater mussels were reduced in metal-contaminated streams that drain the Tri-State Mining District (TSMD) of Missouri, Kansas, and Oklahoma. However, previous laboratory toxicity studies found that juvenile mussels were not among the most sensitive benthic taxa in short-term (4-week) toxicity tests with TSMD sediments. In the present study, we conducted longer-term (12-week) toxicity tests with the fatmucket mussel (*Lampsilis siliquoidea*)and a commonly tested amphipod(*Hyalella azteca)* to establish site-specific thresholds to protect freshwater mussels of the TSMD from injury by toxic metals. Fine streambed sediments (< 2 mm) were collected from depositional areas adjacent to 24 study sites that selected for a concurrent mussel community survey. Sediment toxicity was determined using standard 6-week amphipod tests and using a new protocol for conducting 12-week sediment exposures with juvenile mussels. Both tests included regular replacement of overlying water to maintain water quality conditions similar to TSMD stream waters. In the mussel test, sediments were replaced every four weeks to avoid fouling by food or waste and to allow monitoring of mussel survival and growth (by photography) at each four-week interval. Toxicity thresholds (expressed in terms of sediment zinc or the zinc-lead-cadmium mixture) from the 42-day amphipod tests and 12-week mussel tests were lower than those from previous 28-day tests with these species. Toxicity thresholds for mussels in 12-week tests were 80-90% lower than threshold for previous 4-week tests and were equal to or lower than thresholds from 6-week amphipod tests. These results support our hypothesis that previous short-term sediment toxicity tests substantially underestimated long-term effects of zinc and associated metals on mussels. We are currently investigating whether concurrent exposure of mussel to metal mixtures in stream water result in even lower effective thresholds for toxicity of metals in sediment, and whether lower toxicity thresholds for sediment metals will better predict adverse effects on mussel communities in the TSMD streams.

**395 A Leach Rate Cap on Copper Antifouling Paints in California: A Regulatory Case Study**

*A. Burant, California Department of Pesticide Regulation / Surface Water Protection Program; X. Zhang, California Department of Pesticide Regulation / Environmental Monitoring; N. Singhasemanon, California Department of Pesticide Regulation*

Copper is used as a biocide in boat hull coatings to prevent the biofouling of aquatic vessels. Copper antifouling paints (Cu-AFPs) are the most commonly used biocides in California marinas. A monitoring study by the California Department of Pesticide Regulation (CDPR) found that large saltwater marinas in Southern California typically have the highest dissolved copper (DCu) concentrations. Recreational boat marinas are particularly susceptible to pollution from Cu-AFPs because they are designed to be poorly flushed and are densely packed with seldom-used recreational vessels that have Cu-AFPs applied to their hulls. The monitoring study found that measured concentrations of DCu routinely exceeded national ambient water quality criteria for the protection of aquatic life (i.e., above the chronic and/or acute water quality criterion of 3.1 and 4.8 µg/L, respectively) in many California saltwater marinas. CDPR also measured toxicity in 47 marina samples, eight of which displayed statistically significant toxicity and used the freshwater and saltwater Biotic Ligand Model (BLM) to predict toxicity for the remaining samples. The saltwater BLM predicted toxicity in 18% of the samples. In response to the water quality exceedances and associated toxicity, CDPR developed and employed regulatory mitigation strategies to reduce DCu in marina waters. These efforts were focused on determining a leach rate cap for Cu-AFPs. The cap was developed with aid of the Marine Antifoulant Model to Predict Environmental Concentrations (MAMPEC), which is an integrated two-dimensional hydrodynamic and chemical fate model. Five marina scenarios, ranging in marina dimensions and number of boats, were modeled to determine leach rates necessary to achieve DCu concentrations less than the chronic water quality criteria. Hull cleaning, a major contributor to DCu in marinas, was also incorporated. The selected leach rate of 9.5 µg/cm$^2$/day was based on both risk assessment and pest management efficacy requirements.

**396 Do Constructed Wetlands Remove Metals or Increase Metal Bioavailability?**

*X. Xu, University of Georgia; G.L. Mills, University of Georgia / Savannah River Ecology Laboratory*

The H-02 wetland was constructed to treat building process water and storm runoff water from the Tritium Processing Facility on the Savannah River Site (Aiken, SC). Monthly monitoring of copper (Cu) and zinc (Zn) concentrations and water quality parameters in surface waters continued from 2014 to 2016. Metal speciation was modeled at each sampling occasion. Total Cu and Zn concentrations released to the effluent stream were below the NPDES limit, and the average removal efficiency was 65.9% for Cu and 71.1% for Zn. The metal-removal processes were found out to be seasonally regulated by sulfur cycling indicated by laboratory and model results. High temperature, adequate labile organic matters, and anaerobic conditions during the warm months (February to August) favored sulfate reduction that produced sulfide minerals to completely remove metals. However, the dominant reaction in sulfur cycling shifted to sulfide oxidation during the cool months (September to next March). High concentrations of metal-organic complexes were observed, especially colloidal complexes of metal and fulvic acid (FA), demonstrating adsorption to organic matters became the primary process for metal removal. Meanwhile, the accumulation of metal-FA complexes in the wetland system will cause negative effects to the surrounding environment as they are biologically reactive, highly bioavailable, and can be easily taken up and transferred to ecosystems by trophic exchange.

### 397 Controlled Field Exposures Suggest Modes of Arsenic Accumulation in Adult Eastern Softshell Clams

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Subsistence and recreational shellfish consumption is an important exposure pathway in evaluating human health risks associated with contaminated sediments in the Pacific Northwest. Both natural and anthroprogenic sources of arsenic have led to elevated concentrations in both sediments and shellfish in Puget Sound and is an important contaminant to consider when pursing remedial activities at contaminated sediment sites. Understanding how and from which media shellfish bioaccumulate arsenic is critical to making science-based decisions on whether and how to remediate arsenic contaminated sediments. The purpose of this field study was to understand the uptake of organic and inorganic arsenic from bedded sediments by Eastern softshell clam (Mya arenaria), including understanding the distribution of arsenic in different tissues and the potential for iron amendments to reduce arsenic bioaccumulation. In situ exposures were conducted in two plots located on the west bank of the Lower Duwamish Waterway Superfund Site, Seattle, Washington. Sediment concentrations in these two plots were 30 mg/kg and 10 mg/kg total arsenic. Sediment treatments included homogenized sediment, undisturbed (or non-homogenized) sediment, clean sand, and homogenized sediment with an iron-amendment. Sediment exposures were conducted in six bottomless, perforated 5 gallon buckets that were inserted into the intertidal substrate, allowing for replicated, controlled sediment conditions with freely draining tidal pumping and porewater exchange through the sediment. Six to eight clams were planted in each exposure chamber and exposed for approximately 120 days prior to collection. Results of this study show that bedded sediment had an overwhelming influence on the total body burden of arsenic in clams. Tissue concentrations in the high arsenic treatment were significantly greater than those of the low concentration plot and sand treatment, indicating that the influence of overlying water was limited. Arsenic was found to preferentially accumulate in the siphon skin of clams, with inorganic arsenic concentrations in the siphon skin approximately three orders of magnitude greater compared to the main body. This result corroborates with similar findings published by Oregon DEQ and allows for the management of human-health risk at the site. Iron amendment also shows promise, with decreased tissue concentrations of both total and inorganic arsenic for clams exposed to iron amended sediment.

## Remediation and Restoration: Assessing and Measuring Effectiveness for Contaminated Sediments

### 398 The Pros and Cons of Performance-Based Site Remediation

*G.I. Greenberg, T.A. Verslycke, Gradient*

The goal of hazardous waste site remediation is to reduce unacceptable risks to human and ecological populations potentially exposed to site contamination. Risks to exposed populations are quantified in human health and ecological risk assessments, which serve as the basis for potential risk management actions when unacceptable risks are found. Risk management actions can include removal, engineering controls, and/or site use restrictions *(e.g.*, administrative controls) to reduce or eliminate exposures that were estimated to result in unacceptable risks. To inform remedial extent, preliminary remediation goals (PRGs) are typically calculated assuming a target risk level and relying on the information compiled in the risk assessments. These PRGs are then used as delineation criteria during remediation. In a performance-based remediation, remedial extent is determined in an iterative manner by modeling post-remediation exposures and risks for different remediation scenarios. This allows for the selection of a defined remedial footprint and does not require PRGs or confirmatory sampling in some cases. A performance-based remediation approach can be an effective tool to quantitatively evaluate the estimated benefit (*i.e*., risk reduction) of different remediation option. This approach can also be used to more easily communicate the quantitative impact of key uncertainties identified in the risk assessments on different remediation options. This allows risk managers to select a remedy that is protective of the most sensitive receptors identified in the risk assessment with an understanding of the potential diminishing benefits and increasing uncertainties associated with more extensive remediaton. The pros and cons of a performance-based approach, as compared to a traditional approach, will be illustrated using a project example where soil remediation was warranted due to unacceptable risks from metals in soil to insectivorous mammals and birds. A performance-based approach was used at this site to facilitate communication between the responsible party and the risk manager on selecting the appropriate extent of remediation and circumvent the need for agreement on PRGs and post-confirmatory sampling.

### 399 Is Too Much Wood a Bad Thing? Using Wood Waste Guidance to Improve the Cleanup of Port Gamble Bay

*R. McMillan, J. Evered, L. Machut, WA State Department of Ecology / Toxics Cleanup Program*

Washington’s Toxic Cleanup Program (Department of Ecology) is tasked, in part, with regulating remediation resulting from historic industrial activities. This includes addressing contamination from historic sawmills and wood products manufacturing that occurred throughout Puget Sound over the past 100 years. Wood waste has been a major driver in numerous large scale, nearshore cleanups in Washington State, where its presence contributed substantially to the footprint of the active cleanup and the volumes of sediment requiring remediation. However, cleaning up wood waste is costly and time consuming. Also, the nature of wood waste is highly variable which makes its toxic effects difficult to predict. Unlike many traditional contaminants, there is rarely a simple metric that accurately characterizes effects. A recent cleanup in Port Gamble Bay will be used as a case study to show how the state’s Sediment Management Standards and Wood Waste Guidance can be used to develop a weight of evidence approach, using physical surveys, numeric chemical criteria, and bioassays, to identify areas requiring remediation from overlapping wood waste, PAH, and dioxin contamination.

### 400 Designing and implementing a remedy effectiveness assessment for Thomson and Scanlon Reservoirs in the St. Louis River AOC

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The Great Lakes National Program Office (GLNPO) and the Minnesota Pollution Control Agency (MPCA) are developing remediation and restoration actions for the Thomson and Scanlon Reservoirs on the St Louis River. A multi-Agency team of researchers and project managers from EPA ORD, EPA GLNPO, MPCA, and USGS conducted baseline assessments along multiple lines of evidence using physical, chemical, and biological methods and metrics. The team designed and executed the baseline characterization from 2016-2017 focused on characterizing the two reservoirs for the primary contaminants of concern (COCs) dioxins/furans and mercury. Innovative measures and metrics were used to assess the COCs impacts in the reservoir systems. These included biological metrics such as various macrobenthos measures, spiders, swallows, and multiple fish species. Chemical metrics included surface sediment, sediment at discrete depths, passive samplers, and water measurements of the COCs. Finally, physical measures included bathymetric surveys and sediment physical characterization. The results of these assessments will be presented and compared to a reference condition.

**401 Influence of remediation on sediment toxicity within the Grand Calumet River, Indiana**

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The Grand Calumet River (GCR), located in northern Indiana, is contaminated due to a wide range of historical industrial activities. This study was conducted to determine the influence of sediment remediation within the GCR on the levels of sediment contaminants and toxicity to sediment dwelling organisms. Sediments with high concentrations of metals and organic compounds were remediated through a combination of removal, capping, and activated carbon amendments between 2005 and 2016. A combination of acute and chronic sediment toxicity tests were conducted three times from 2013 to 2017 using the amphipod *Hyalella azteca*, the midge *Chironomus dilutus*, and the mussel *Lampsilis siliquoidea*, in sediments from 29 sites, including both remediated and non-remediated sites. Previous studies focused on short-term (10-d) exposures to evaluate the severity of sediment contamination at the site, with results of longer-term (28- to 42-d) exposures conducted to determine if sediments with lower concentrations of contaminants after remediation were still toxic. Reduction in survival and biomass was observed for all sediments across the GC although the level of toxicity varied from year-to-year. The chemistry and bioassay lines-of-evidence were evaluated using a scoring approach and evaluated across three regions of remediated sites and compared to non-remediated locations. Toxicity data for each year and sample location was scored based on the number of toxicity endpoints that were significantly reduced relative to the control. Temporal trends in toxicity scores, such as such as year-to-year variation, indicate there is a wide range of toxicity in both remediated and non-remediated sites that may be due to other site-specific factors associated with the site. Site specific factors such as recontamination due to combined sewer outfalls and other sources will be discussed as potential conditions affecting remedy success and long term stability.

**402 PCB export at the lower Ottawa River (Maumee River AOC): An analysis of sediment, passive samplers, aquatic invertebrates, and riparian spiders**

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Environmental dredging at select site locations was performed within the lower Ottawa River, a portion of the Maumee River Area of Concern (Ohio, USA), in 2010 to remediate sediments contaminated with PCBs, PAHs, and lead. Prior to dredging, we designed a multi-metric study to assess contaminant fate and transport, ecosystem response, and recovery using a multiple lines of evidence approach. We measured PCB concentrations in surface sediment, passive samplers deployed in the water column, aquatic invertebrates, and spiders from 2009-2013 & 2015. Site-specific PCB concentrations were significantly and positively correlated among all metrics across all years except for sediment. Sediment concentrations at remediated and non-remediated sites were highly variable throughout the study period and were unreliable indicators of PCB concentrations in biota. PCB concentrations in aquatic invertebrates were highly correlated with concentrations measured in passive samplers, and in turn, were strongly predictive of concentrations in riparian spiders that are specialized predators of adult aquatic insects. All of these measures of PCB uptake, accumulation, and trophic transfer declined by 2015, indicating that the dredging remedy was successful in reducing PCBs concentrations in biota within the Area of Concern.

**403 An Independent Evaluation of the Hudson River PCB Dredging Program**

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In February 2002, the General Electric Company (GE) was ordered by the U.S. Environmental Protection Agency (EPA) to conduct targeted dredging of PCB-contaminated sediment in a 40-mile stretch of the Upper Hudson River between Fort Edward and Waterford, NY. GE performed dredging of the Upper Hudson in two phases, beginning in May 2009 and ending in October 2015. Following completion of dredging operations, the Hudson River Foundation convened an expert panel to evaluate the effectiveness of the dredging program on the Upper and Lower Hudson. Based on water column and fish monitoring data (through December 2016) and simple model calculations, the panel concluded: (i) the dredging program met mass removal targets for PCB-contaminated sediments, (ii) the dredging program was effective in reducing PCB concentrations in fish from Thompson Island Pool, (iii) post-dredging PCB concentrations in fish downstream of Thompson Island Pool showed mixed results, (iv) the reduction in Tri+ PCB loads to the Lower Hudson during the 2016 post-dredging period were in part due to below-average flows in the river, (v) water column, sediment and fish in the Lower Hudson below Albany are showing slow responses to the Upper Hudson dredging program due to the complexities of sediment transport in the Lower Hudson, and (vi) additional years of natural attenuation will be required to reduce PCB concentrations in fish throughout the Upper and Lower Hudson to acceptable levels. Modifications to the post-dredging monitoring program and continued evaluation of the next few years of monitoring data are therefore recommended to assess if natural attenuation will be sufficient in reducing PCB concentrations in fish in a reasonable time frame or if additional remedial actions will be required. The panel's evaluation is currently being extended to include 2017 monitoring data, which corresponds to the second year of the post-dredging period.

**404 Retrospective Review of Long-Term Biological Metrics to Assess Completed Sediment Cleanup Remedy Effectiveness**

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Contaminated sediments pose unique challenges, both technically and administratively. Throughout North America, governmental agencies and responsible parties have been investigating, evaluating, and remediating contaminated sites since the early 1980s under a range of regulatory frameworks. Although the objective of sediment remediation is to reduce potential risks to human health and the environment, unfortunately it is still not clear whether the engineering approaches in most widespread use effectively reduce such risks. Even today, the scientific and engineering community seeks effective approaches to manage contaminated sediment risks. The high costs associated with sediment remediation, coupled with uncertain risk reduction outcomes, have led many practitioners to redouble efforts to collect, learn from, and incorporate new information to improve the effectiveness of remedial operations. Using long-term contaminant bioaccumulation and histopathology monitoring records available for indicator fish species along with other available biological metrics, a retrospective review of the effectiveness of completed large-scale sediment cleanup remedies in the U.S. was initiated. Results of the review to date reveal that the effectiveness of sediment remediation depends in large part on whether surface sediment concentrations are attributable to legacy inputs or the result of ongoing sources. Where ongoing sources control surface sediment concentrations, active remediation of sediments in the absence of additional source control has been ineffective. In these cases, source control has been shown to be effective in accelerating natural recovery of biological metrics. Moreover, exposure control to achieve desired fish tissue recovery has been principally achieved by capping or backfill regardless of whether environmental dredging was undertaken. Ongoing remedy effectiveness evaluations will be summarized.

**405 Assessment of Human Health Benefits and Risks of Contaminated Sediment Remediation**

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Billions of dollars have been spent on environmental dredging projects to remediate contaminated sediments, with billions more slated for this; however, the extent to which this remedy can reduce human health risks needs to be quantified on a population scale and compared with potential induced risks. This study is the first to do so, focusing on the Hudson River Polychlorinated Biphenyls (PCBs) Superfund Site remediation (2009-2015). By utilizing a combination of mechanistic modelling, monitoring, and survey data, we quantify and compare the potentially avoided and induced population health burden of this major dredging project in terms of disability-adjusted life years (DALY) with approximate 95% confidence intervals (CI). The specific aims were as follows: 1) Assess the potential cancer and non-cancer health burden on recreational anglers for a No Action scenario due to bioaccumulation of PCBs in Hudson River fish and exposure through fish consumption. 2) Determine and compare long-term reductions in health burden from reduced fish tissue PCB concentrations under different remedial alternatives, including resuspension, relative to No Action. 3) Investigate the potential health burden induced by the selected dredging remedy from increased air emissions of PCBs, $PM_{2.5}$, and $NO_x$, and fatal occupational incidents. 4) Compare the avoided burden (benefits) with the induced burden (risks). For the No Action scenario, the central estimate health burden attributable to bioaccumulation of PCBs in Hudson River fish and exposure through fish consumption is 11 DALY. Implementing a source control action under Monitored Natural Attenuation (MNA) achieves an avoided burden of 4 DALY (CI 0.06 to 321 DALY) over No Action, while dredging achieves an additional 1 DALY (CI 0.02 to 102 DALY) of avoided burden. The estimated total burden induced by dredging is 40 DALY (CI 7 to 148 DALY), which is dominated by rail transport of PCB waste across the US (25 DALY, CI 4 to 153 DALY), and fatal occupational incidents (14 DALY, CI 0 to 88 DALY). Future decisions on the need for removing contaminated sediments should thus better weigh health risks associated with dredging activities.

## Developments in Water Quality Monitoring and Analytical Methods in Support of Water Reuse – Part 1

**406 High throughput monitoring of glyphosate in the Lake Erie Basin using IC-ICP-MS: Method development and preliminary data**

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Glyphosate is a broad spectrum, non-selective herbicide that is widely used in agriculture and urban watersheds. According to National Agriculture Statistics Service, glyphosate use in agriculture in the state of Ohio has increased over 20 times from 150 metric tons in 1990 to 3100 metric tons in 2015. Research in Lake Erie watersheds has found glyphosate concentrations in the Maumee River and the western basin of Lake Erie ranging from 0.07 to 1.57 µg/L. While generally considered nontoxic at these levels, some research has indicated that glyphosate could play a role in increasing of dissolved reactive phosphorus loadings from farm fields since the mid-1990s by out-competing phosphate for sorption sites in soil. Apart from the Great Lakes region, detectable concentrations of glyphosate have been found in streams from different parts of the world including the mid-western US, Canada, Argentina, and Switzerland, with concentrations ranging from 0.5 to 70 µg/L.Due to its increasing use throughout the world, and potential ecological risks, new high throughput methods are needed to better understand and characterize runoff patterns in agricultural and suburban watersheds. There are few existing methods that can rapidly measure glyphosate and its primary degradation product aminomethylphosphonic acid (AMPA) at environmental concentrations. We present a newly developed method for glyphosate and AMPA analyses using direct injection ion chromatography inductively coupled plasma mass spectrometry (IC-ICP-MS ) with a detection limit of less than 1 µg/L. This method requires little sample preparation, no derivatization, and provides high sensitivity at low cost. The method provides a good alternative to more elaborate procedures requiring derivatization. To demonstrate the utility of this method, preliminary data on Lake Erie Basin runoff water samples will be presented.

**407 Highly Sensitive Detection of Trace Organic Contaminants in Water Using Automated Online SPE and a Novel Triple Quadrupole LCMS System**

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A variety of organic contaminants originating from personal care products, pharmaceuticals, hormones, pesticides, and industrial chemicals are introduced into the environment through domestic and industrial wastewater. These molecules are collectively known as the trace organic compounds (TOrCs) and commonly detected in a variety of resources. Although not acutely dangerous individually, long-term synergistic exposure due to mixtures of these compounds are still to be determined. While studies on long-term/chronic toxicity are yet to be published, it is very important to accurately monitor their presence in environments at trace levels. This requires a methodology to quantify TOrCs with high sensitivity and throughput. Automated online SPE solution fit in well and provides advantage to enrich the analytes for better sensitivity. With minimal human intervention, it improves the throughput of testing lab and measurement precision. TOrCs captured on polymer-based PLRP cartridge were eluted using a linear gradient onto a reversed phase C-18 analytical column for separation and detection. The novel LC-MS/MS is designed to address many challenges faced by routine production labs and high-throughput academic labs. Innovative technologies within the instrument allowed for reduced overall footprint, while conserving the comparable performance level. Innovations, such as VacShield, reduces the need for user intervention for system maintenance. Further, the use of online SPE significantly lowers sample volume, solvent usage while improving robustness and ease of use. Our results demonstrated that most of the analytes could be detected at 1 ng/L with 900uL injection after filtration through a 0.2-µm syringe filter. Samples were analyzed using Agilent Jet Stream electrospray ionization with fast polarity switching. Dynamic MRM (dMRM) transitions for 51 analytes were optimized using an automated tool in MassHunter Acquisition software. Method performance was evaluated using method detection limit (MDL) as metrics of overall sensitivity. The method precisions were calculated based on 6 replicate injections of spiked standards and measurement accuracy has to be within 80-120% for at least 4 of the replicates. Most of the analytes had a %RSD less than 15%.

**408 Rapid Low-Volume Method for N-Nitrosamine Analysis in Recycled Water for Benchtop or Online Monitoring**

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Current methods for analysis of N-nitrosodimethylamine (NDMA) and other N-nitrosamines require laborious liquid or solid phase extraction followed by gas or liquid chromatography coupled with dual mass spectrometry. The National Research Council recently highlighted the importance of NDMA in recycled water due to its occurrence in treated wastewaters at levels closest to levels of potential human health concern. As such, potable water reuse plants in California are required to monitor NDMA in their finished water. Due to the labor and high equipment costs, NDMA analysis is challenging for many utilities. A novel method for analysis of NDMA and three additional N-nitrosamines in drinking water that requires less time, cost, and sample volume was developed in 2009 and validated in the present study for recycled water. The method requires only a 200 µL direct injection for high-performance liquid chromatography (HPLC) separation and thus the need to collect large volumes of sample (typically 0.5 to 1 L for similar methods) and perform sample extraction is abated. The method uses a UV reactor and chemiluminescence detector to measure N-nitrosamine breakdown products and was validated using a prototype instrument at Orange County Water District following validation criteria from EPA Method 521 for N-nitrosamine analysis. Accuracy and precision for four N-nitrosamines (NDMA, N-nitrosomorpholine, N-nitrosopyrrolidine and N-nitrosomethylethylamine) was acceptable using these criteria. The method reporting limits for the four N-nitrosamines were 1.2, 1.8, 5 and 5 ng/L, respectively, which are equal to or better than most conventional N-nitrosamine methods. The accuracy and precision of the method was also determined for various water matrix types including secondary wastewater effluent, microfiltration (MF) effluent, and finished product water after full advanced treatment for potable reuse. Significantly, this method has been adapted to enable automated online, near real-time NDMA analysis in a pilot treatment system (measurement of NDMA every ~20 minutes) and thus has potential for improving monitoring frequency at recycled water facilities. This may have particular benefits for "direct" potable reuse facilities in which finished water is served soon after treatment.

**409 Assessment of nitrosamines in drinking water using GC-MS/MS with a novel Electron Ionization source**

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A gas chromatography tandem mass spectrometry (GC-MS/MS) method for determination of seven N-nitrosamines (NAs) in drinking water is described that utilizes a novel electron ionization source. Nitrosamines are emerging drinking water contaminants linked to cancer by either ingestion or inhalation. Due to their toxicity, nitrosamines are considered as priority pollutants and various countries around the world have already introduced maximum acceptable levels for instance in EPA 521.1. Large volume injection (20 µL), chemical ionization followed by selected ion storage (SIS) resulted as the mass spectrometric method of choice, with detection limits in the range of 1-2ng/L however chemical ionization has problems with robustness and SIS has a narrow linear dynamic range. Solid Phase Extraction (SPE) with coconut charcoal cartridges was applied to extract NAs from 17 real samples collected from different drinking water treatment plants and their distribution networks. In this analysis using a 2 µL injection excellent sensitivity was observed down to 0.003 pg on column (low ppq, w/v) in sample. Outstanding linearity over a range of 0.05-20 pg/µL corresponding to 0.05-20 ng/L in sample with $R^2$>0.999 and RRF % RSD < 5%. Method detection limits were calculated at 0.008-0.045 ng/L. The LOQ was established at between 0.1-0.5 ng/L for all NAs and excellent recoveries were obtained with all values between 70-130% with an average recovery of 93%. The total quantitated nitrosamine amount in the seventeen drinking water samples ranged between 0.9-4.5 ng/L. Here the use of a novel electron ionization (EI) source in conjunction with a triple quadrupole mass spectrometer provides the sensitivity and selectivity to analyze nitrosamines in drinking water at low part per trillion (ppt) levels with low liquid injection volumes and without the requirement for SIS or chemical ionization.

**410 Development of comprehensive libraries for water quality monitoring: The case of per- and polyfluoroalkyl substances (PFASs) in commercial products**

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Per- and polyfluoroalkyl substances (PFASs) are highly persistent and used widely in industrial and consumer applications (e.g., fire extinguishers, non-stick pans, and food packaging). Municipal and industrial run-off has been suggested to be the main source of PFASs. Over 4730 PFASs with CAS number are commercially available, yet due to analytical limitations, research on the fate and effects of PFASs typically focuses on a small subset of chemicals. Several PFASs have been detected ubiquitously in the environment and may be linked to cancer, elevated cholesterol, and immune suppression. Industries have adapted with new structures of some emerging PFASs (e.g., shorter carbon perfluoroalkyl chains) render these compounds more mobile and difficult to treat. The historical approach of measuring individual compounds in these products and the environment is an inefficient way to identify problematic emerging PFASs. Fortunately, advances in analytical techniques, specifically High Pressure Liquid Chromatography Quadrupole Time of Flight Mass Spectrometry (HPLC-Qtof-MS/MS), now enables identification of thousands of organic substances in complex chemical mixtures that make up commercial products or environmental samples. The goals of this study were to build a comprehensive Personal Compound Data Library (PCDL) with spectra from multiple sources and to challenge the library with samples of aqueous film forming foam, which are suspected to be important sources of PFASs to the environment. The PCDL was constructed with 3480 chemicals from multiple sources including in house standards, MassBank, MZCloud, the Norman Network PFAS suspect list, literature reported fragmentations and theoretical fragmentations. The method qualified 11 PFASs with various structures in the samples of aqueous film forming foam (AFFF) produced in 1999 by 3M company. The presentation will highlight opportunities and challenges of suspect screening approaches for monitoring trace organic chemicals. Most studies continue to focus on investigating one or a select suite of compounds at a time due to limited resources for commercial standards, a large number of candidates, lack of effective qualifiers, inefficient fragmentation, matrix effects, and small linear windows for calibration curves. Broad screening techniques are expected to provide value in the detection of whole classes of chemicals like PFAS in complex aqueous mixtures such as wastewater effluent and recycled water.

**411 Wastewater Reuse and Chemical Contaminants of Concern: Understanding Water Quality and Community Perceptions**

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The city of Jacksonville, NC, uses a forested land treatment site (LTS) to reuse its municipal wastewater by applying secondary treated effluent to forest soils via slow-rate irrigation. Although such forested systems have been utilized for decades in the US, they have only recently been investigated in terms of their roles as a source of emerging contaminants into the environment. Population growth and limited land-expansion opportunities are now driving discussions for alternative means to expand the capacity of the Jacksonville LTS, with the potential for wastewater application to surrounding agricultural land. Our project goals are to characterize the potential human health risks of chemical contaminants of concern in land-applied wastewater at the Jacksonville LTS and to better understand community values and perceptions of alternative wastewater reuse to agricultural lands. Preliminary data from suspect-screening and targeted chemical analyses of water samples indicate the input of organic chemical contaminants at the LTS from wastewater into surface and ground water. Studies of such sites in other areas of the country have demonstrated that citizens often have concerns about their lack of involvement in decision-making processes and health and safety issues regarding wastewater reuse; therefore, survey data obtained from a Community Involvement Group (CIG) provides us with information on local perceptions and values regarding current, non-traditional wastewater use and risks, as well as options for alternative water reuse in the future. Overall, the CIG will work together with the project's leadership team to formulate appropriate strategies to communicate project findings to the broader public.

**412 Potable Reuse and Microbial Risks – a Critical Review and Comparison of Risks between Planned and De Facto Reuse Scenarios**

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There is an increasing interest in recycling water for potable reuse purposes which makes it important to understand the associated microbial risks. To better understand these risks, we conduct numerical simulation analyses to compare risks from a series of representative *de facto* reuse, indirect potable reuse (IPR) and direct potable reuse (DPR) scenarios using a previously published QMRA methodology and literature review results. Variables in the analyses included: (for *de facto* reuse) the percent wastewater contribution in surface water and the environmental residence time in days; (for IPR) advanced water treatment facility (AWTF) treatment train choice, the percent AWTF contribution to the source water, and time between blending and drinking water treatment in days; and (for DPR) AWTF treatment train and relative level of disinfection for DPR. Additionally, the *de facto* reuse simulation results were compared to a *Cryptosporidium* spp. database collected for the Long Term 2 Enhanced Surface Water Treatment Rule's Information Collection Rule (ICR) and to a systematic literature search of norovirus (NoV) densities in ambient surface waters. The *de facto* simulation comparison revealed that a wastewater contribution of 1% in surface waters and a residence time of 90-days most closely match the ICR dataset. These conditions were then modeled for IPR simulations. Additionally, the simulation comparisons suggest that use of NoV data collected from ambient waters may overestimate microbial risks, in contrast to the use of NoV data from raw sewage. Overall, the simulated predicted risks from IPR and DPR scenarios were consistently lower than those for the *de facto* reuse scenarios. Collectively the analyses provide additional insight about the microbial risks associated with various potable reuse scenarios and highlight the need to carefully consider drinking water treatment choices when wastewater is a component of any drinking water supply (whether *de facto*, IPR, or DPR).

**413 Flow cytometry for rapid detection, enumeration, and characterization of waterborne viruses**

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The ability to reliably monitor waterborne viruses is essential to ensuring safe water treatment, recycling, and reuse. Waterborne viruses pose significant direct health risks. Moreover, because removal requirements are higher for viruses than for other waterborne pathogens, monitoring virus removal is a conservative approach to assessing the effectiveness of water-treatment processes. Unfortunately, conventional methods of virus monitoring are limited by low sensitivity, extended time needed to obtain results, and numerous other factors. Flow cytometry (FCM)—the analysis of particles based on how they scatter light and fluoresce when passing through a laser beam—offers an alternative approach. Recent advances in FCM have made it a viable tool for water-quality monitoring. Numerous researchers have demonstrated the value of FCM for analysis of waterborne bacteria. This study examines applications of FCM for rapid detection, enumeration, and characterization of waterborne viruses (also known as flow virometry). Specifically, we optimize protocols of FCM-based analysis of four strains of indicator bacteriophages (T4, x174, 6, and MS2) representing a range of sizes, morphologies, and nucleic-acid structures. We then test the optimized protocols on spiked samples of environmental waters (tap water, river water, rainwater, and wastewater taken from three treatment stages). The results build the case for incorporating FCM as a routine monitoring approach in a variety of water treatment and reuse scenarios, including direct potable reuse.

## Fate and Effects of Chemicals from Diffuse Sources and Stormwater – Part 1

**414 Science-based regulatory approaches for addressing stormwater pollution from diffuse sources**

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Traditional water pollution regulatory approaches, such as those embodied in the US Clean Water Act, stem from the assumption that pollution comes from large facilities like major industries. Due in part to the success of traditional regulatory programs – and in part to the increased use of specialty chemicals in ordinary products – the main sources of water pollution have shifted to "diffuse sources" like consumer products and pesticides. These shifts demand a shift in water quality regulatory approach. Government agencies in the state of California are responding to this challenge through three major regulatory programs. Scientific research provides key data for these programs by identifying pollutant sources, demonstrating linkages between pollutant sources and surface waters, and detailing the environmental impacts of diffuse pollutants. California Department of Toxic Substances Control's (DTSC's) Safer Consumer Products Regulatory Program is currently piloting its authorities to protect aquatic ecosystems. In May 2018, California municipalities petitioned DTSC to address zinc in vehicle tires (one of two major zinc sources in urban runoff). These California agency efforts are informed by the practical experience and the science behind the first major US regulatory program to address a diffuse water pollution source (copper in vehicle brake pads). Using science linking pesticides application methods to urban runoff loads, California Department of Pesticide Regulation's Surface Water Protection Program has adopted regulations addressing urban runoff pesticides pollution and has expanded scientific review of new pesticide registration applications. California State Water Resources Control Board's Strategy to Optimize Resource Management of Storm Water proposes to integrate California and Federal pesticides regulatory authorities into its urban runoff Clean Water Act compliance program. This change could speed cleanup of pesticides water pollution while saving California state and local governments millions of dollars. Ultimately, better informed product design could avoid water pollution. Many major corporations now voluntarily integrate chemicals safety screening into the early phases of product design. Current screening methods have limited capacity to address diffuse urban runoff pollution. Additional scientific information – and better predictive modeling tools – have the power to inform a clean water future.

**415 Roads to ruin: The threats of urbanization to conservation of a sentinel species**

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Since the late 1990s, coho salmon adults returning to their natal urban streams in Puget Sound experience high rates (e.g., 40-90% of run) of spawner mortality syndrome. Evidence suggests that urban stormwater runoff is the likely causative agent and that this high mortality may threaten wild coho populations, particularly in urbanizing basins. The ability to identify basins currently at risk for this syndrome is critical to conservation efforts. We looked at the relationship between the mortality syndrome (time series of coho spawner survey data from 51 streams distributed across an urban gradient in the Puget Lowlands) and basin scale habitat conditions (time series of nationally available geospatial data, including seasonal precipitation) to better understand the landscape characteristics most associated with the syndrome and to generate predictive maps of mortality rates in unmonitored basins. Structural equation modeling revealed a latent urbanization gradient that was associated with road density and traffic intensity, among other variables, and positively related to mortality, which is consistent with other studies that suggest motor vehicles are the likely source of a chemical mixture that washes off urban landscapes into coho spawning streams. Across years within sites, mortality increased with summer and fall precipitation, but the effect of rainfall was strongest in the least developed areas and was essentially neutral in the most urbanized streams. We used the best-supported structural equation model to generate a predictive mortality risk map for the Puget Sound Basin. The predictive mortality map identified likely hotspots for coho spawner die-offs in unmonitored basins across the gradient of urbanization in Puget Sound. Our analyses improve our understanding of the interplay between urbanization and climatic drivers of the mortality syndrome, are easily transferable to other regions, and can be used for siting green stormwater infrastructure in the current built environment and in future development scenarios.

**416 Assessing Acute Sublethal Effects of Stormwater Runoff on Coho Salmon**

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Urban stormwater runoff poses a direct threat to the health of aquatic ecosystems. In the Pacific Northwest, coho salmon (*Oncorhynchus kisutch*) are recognized as a sentinel species for stormwater impacts due to their acute lethal response. Research to date has been directed towards understanding and preventing lethal impacts of urban runoff to coho salmon. In direct exposures to road runoff, coho experience 100% mortality within 24 h. The current study aims to determine what dilutions of road runoff are necessary to prevent mortality and to assesses potential sublethal effects on coho salmon exposed to urban runoff. Pilot experiments show mortality occurs within the first 24 h of exposure and that dilutions of >90% are necessary to prevent mortality. Continuing research will use stormwater dilutions to assess sublethal impacts to coho exposed to runoff, including DNA damage, hormone imbalances and gene expression patterns. Indicators of sublethal physiological stress in coho could imply threats to survival and reproductive success at stormwater concentrations much lower than previously hypothesized. Gaining a comprehensive understanding of stormwater toxic effects is critical for implementing appropriate regulatory actions to protect coho salmon and other organisms exposed to urban runoff.

**417 Tire leachate recapitulates the pathophysiology, unique sensitivity, and mortality of coho salmon acutely exposed to urban road runoff**

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Coho salmon (*Oncorhynchus kisutch*) spawners returning to spawn in streams receiving urban road runoff suffer high rates of pre-spawning mortality. Direct exposure of spawners to road runoff causes a significant loss of blood ions, drop in pH, and increase in hematocrit, followed by death within hours of exposure. Neither the pathophysiology nor the mortality is seen in concurrently exposed chum salmon (*O. keta*). Contaminants in road runoff are dominated by vehicles sources including fluid leaks, and particulates from exhaust emissions, brake friction materials, and tire wear. Chemicals that leach from tires or tire particles can be acutely toxic to aquatic animals including fish. We tested whether particles from tires could leach sufficient toxic chemicals into water to be acutely lethal to coho salmon. Fine tire particles were generated and placed in a 100-mm mesh filter sock, inserted into an HDPE filter housing, and continuously leached with a fixed volume of clean well water for 22 h. The resulting filtrate (leachate) was essentially clear and devoid of visible particles. Adult coho salmon returning from Puget Sound were placed in well water or a dilution of well water and tire leachate. The lowest concentration that killed all coho spawners within 5 h (0.32 g/L) was used for subsequent testing. At this concentration, all coho exposed to the tire leachate died within 24 h whereas none of the exposed chum spawners died. Finally, blood sampled from coho exposed for 3 h to 0.32 g/L tire leachate showed significant loss of Na and Cl, drop in pH, and increase in hematocrit whereas the blood of chum concurrently exposed did not show these changes. Tire particles may be an important source of the unknown chemicals contributing to pre-spawning mortality in coho salmon exposed to urban road runoff.

**418 Screening of stormwater runoff for environmental contaminants following wildfires in Northern California**

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Stormwater runoff picks up pollutants that are present on the landscape during dry days and transports it to downstream areas. It is widely known as a major source of environmental contaminants. In late 2017, wildfires ripped through Northern California's Sonoma, Napa and Mendocino counties, affecting > 115,000 acres of land and destroying ≈ 7000 structures across the state. To evaluate the possible effects of these fires on the types of pollutants in stormwater, and subsequently the ecological impact in San Francisco Bay and the coastal marine environment, we monitored stormwater collected after the wildfires using a non-targeted analytical (NTA) approach. A total of 25 stormwater samples were collected from Napa, and Sonoma counties during two storm events in November 2017 and January 2018. Samples were extracted using solid phase extraction on Hydrophilic-Lipophilic Balance (HLB) and Weak Anion-eXchange (WAX) cartridges. Instrumental analysis was carried using Agilent's 6550 liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (LC-QToFMS) in both positive and negative electrospray ionization (ESI) modes. Identification and confirmation of compounds was facilitated using suspect and non-target screening techniques on Agilent's Masshunter Qualitative Analysis, Profinder, Mass Profiler Professional and Personal Compound Database and Library Manager (PCDL) software. Our in-house databases and libraries contain a wide range of compounds including per- and polyfluoroalkyl substances (PFASs), pesticides, organophosphate flame retardants (OPFRs), and a partial list (≈ 1300 compounds) of a wide range of compounds found on EPA web-based CompTox Chemistry Dashboard. Preliminary screening results tentatively identified the presence of a range of compounds including herbicides and substances present in consumer products. We will present details of the NTA workflow used in the identification of the compounds detected, and an in-depth comparison between the results from the different counties over the two rain events. In addition, we will compare the time series samples to monitor any significant attenuation in contaminants from the initial storm water samples collected after the wildfire. The views expressed herein are those of the authors and do not necessarily reflect those of the California Department of Toxic Substances Control.

**419 Statistical Modeling and Sampling Design Optimization for Monitoring Pollutant Trends in San Francisco Bay Urban Stormwater**

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The Regional Monitoring Program for Water Quality in San Francisco Bay (RMP) conducts stormwater loadings studies at small tributaries in the San Francisco Bay region. Here, a statistical framework to optimize the sampling design for monitoring of trends in loads of pollutants of concern at small tributary sites was developed. The longest-running time series of tributary monitoring for polychlorinated biphenyls (PCBs) on the Guadalupe River (2003-2014) was selected as the case study for this work. The statistical approach builds upon the turbidity surrogate methodology that has been employed in tributary monitoring by the RMP since 2003. A two-stage statistical modeling technique was developed to incorporate the significant turbidity-PCB relationship that exists, and evaluates climatic, seasonal, and inter-annual factors as additional potential drivers of PCB loads. After accounting for climatic factors related to antecedence and local watershed precipitation in the statistical model, no significant linear inter-annual trend in PCB loads for the period 2003-2014 was found. Subsequent power analysis of the statistical approach indicated a discrete-based sampling methodology could achieve > 80% power to detect linear declines of 25% or greater in PCB loads, over a 20-year period. Analysis of composite-based sampling designs indicated that this approach was less sensitive to trends, and could only detect larger trends (>75%) over 20 years. Overall, the results of this work suggest that statistical models can be used to control for climatic variation in PCB loads from watersheds, such that trends can be better detected using traditional discrete sampling methods. To further expand this work to a broader context will require monitoring in additional watersheds to test if the proposed approaches to improve trend detection are more widely applicable in San Francisco Bay.

**420 An Analysis of Chemical Contaminants in Sediments and Fish from Cocos Lagoon, Guam**

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The US Coast Guard operated a Long Range Navigation (LORAN) station on Cocos Island at the southern end of Cocos Lagoon, Guam, from 1944 to 1963. Disposal of materials from the operation of the station are suspected of resulting in chemical contamination of the island and surrounding waters. To help Guam EPA address this, the NOAA/NOS National Centers for Coastal Ocean Science (NCCOS) collected sediment and fish samples for chemical contaminant analysis throughout Cocos Lagoon in May 2015, with local partners. Results of the analysis indicated low levels of most chemical contaminants in sediments. The organochlorine insecticide DDT was somewhat elevated in sediment on the northern end of Cocos Island, exceeding a NOAA sediment quality guideline. Elevated levels of polychlorinated biphenyls (PCBs) along with the pesticide DDT, however, were found in most fish collected adjacent to Cocos Island. A number of the fish contained concentrations of both PCBs and DDT above subsistence and recreational fisher Screening Values established by the US Environmental Protection Agency. Lower concentrations of PCBs and DDT were found in fish from other areas of Cocos Lagoon, however, some were above the subsistence Screening Value for PCBs. Additional sampling using passive water samplers around the island is in progress to assess whether PCBs and DDT are being transported in the water column either as a result of surface water runoff, or through groundwater inputs from Cocos Island that could subsequently be taken up by fish and other marine organisms in the area.

**421 Total Aluminum: Not Totally Relevant for Water Quality Standards**

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In a large dataset spanning 10 years and more than 100 surface water sampling locations on the Pajarito Plateau, New Mexico (NM) aluminum (Al) concentrations were evaluated in unfiltered and filtered samples (10, 1, 0.45, and 0.2 mm filters). Observed Al concentrations often exceeded ambient water quality criteria (AWQC) regardless of filter size and sample location. Al concentrations upstream and downstream of developed areas within and around Los Alamos National Laboratory (LANL) were similar. Solubility calculations showed that most Al concentrations were over-saturated with respect to amorphous $Al(OH)_3$(s), regardless of filter size. EPA 1988 and 2017 proposed Al AWQC are based primarily on total Al effect concentrations calculated from toxicity tests using soluble Al salts. Potential contributors to observed toxicity in these AWQC toxicity databases are only dissolved (ionic) or freshly precipitated (e.g., amorphous $Al(OH)_3$(s)) forms of Al. In contrast, most natural waters would rarely be expected to contain amorphous $Al(OH)_3$(s), but would frequently be expected to contain aluminosilicates – a stable mineral form of Al that is not bioavailable, but can be present in particulates smaller than 1 mm. In the current dataset, total Al concentrations in wet weather samples were strongly associated with elevated suspended sediment concentrations, suggesting that aluminosilicates contribute to AWQC exceedances. Spectroscopic analyses verified aluminosilicate particles in 1-mm filtrates. While 10-mm or 0.45-mm filtrates yielded fewer AWQC exceedances than total Al measurements, evidence indicates aluminosilicates were still retained in filtrates and contribute to exceedances. In order to more accurately measure AWQC exceedances, preparation of environmental samples should differentiate bioavailable and non-bioavailable Al forms. Because size ranges of amorphous $Al(OH)_3$(s) and aluminosilicates overlap, a sample preparation protocol that solubilizes amorphous $Al(OH)_3$(s), but that does not solubilize aluminosilicates, could be used prior to filtration. However, filtration with 0.45-mm filters may still retain non-bioavailable aluminosilicates in environmental samples, and depending on the particle size distribution, smaller pore size filters may be needed. Further development/validation of such criteria implementation protocols is necessary. This uncertainty overshadows numerous 303(d)-listed waters impaired by total recoverable Al and Al TMDLs already completed.

## Dealing with Bias in Environmental Research: Case Studies and Practical Strategies

**422 Biases and Conflicts of Interests in Environmental Science: Development of Practical Solutions**

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The National Academy defines bias as "… positions taken that are largely intellectually motivated or that arise from the close identification or association of an individual with a particular point of view…" It is well-recognized that biases are present in science, regardless of sector and that they can be introduced anywhere in the scientific process from project development, data collection and assessment to publication and peer review. Social, ethical, and financial influences often enter into our decisions; but, as ethical scientists, we attempt to minimize biases. While there are many practical solutions to increasing transparency and minimizing bias (e.g., publication of raw data, double-blind peer reviews, involvement of 'neutral' third parties), few have gained traction to date. Until ideas for reducing biases are introduced and gain wider acceptance, few will be willing to proactively do what it takes to affect change. This presentation as well as others in this session build upon previous SETAC North America sessions (two in Salt Lake City in 2016 and one in Minneapolis in 2017). We have developed this session to provide a platform for continued dialogue and development of practical solutions to minimize bias in environmental science.

**423 Study Design Bias or Bias by Design: Impact of Measurement Endpoint Bias**

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Evolution of higher-tiered, modern-day toxicological bioassays has led to the development of technically-sophisticated measurement endpoints to support interpretation of results produced. Development and incorporation of these endpoints into test designs is critical in support of the highly specific objectives of these assays. Because many of the endpoints incorporated into the design are highly focused and the number of endpoints needed to provide evidence to support a response is often substantial. Measurement endpoints capable of discerning specific effects, such as various aspects of endocrine disruption, are selected over endpoints which would assist in discriminating against overt toxicity or toxicity induced by an alternative mechanism. Incorporation of a more balanced set of measurement endpoints would alleviate issues in interpretation of results resulting in ambiguous conclusions. Two case studies in which additional endpoints assisted in clarifying results and conclusions drawn will be discussed. Ultimately, these case studies demonstrate that consideration of the measurement endpoints used at the study design level reduced measurement endpoint bias and ultimately impact on interpretation bias in the conclusions drawn.

**424 How do you know: When does bias against uncertainty get in the way of making a decision?**

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Bias in risk assessment has been attributed to the varying perspectives and goals of individuals and their organization or sector. Indeed, offices or agencies even within a sector can have different missions (e.g., protection of listed species, sustainability of food production) and individuals are charged to follow the mission of their respective agency. This is not wrong, and individuals should advocate for the mission that they signed up to achieve. By their nature, properly designed and executed scientific studies are unbiased, as the scientific method controls for factors that would introduce bias and isolates the relationship of an endpoint to measurable factors. It follows that the role of peer reviewers is to ensure that studies are properly designed and conducted in such a manner that, once published, scientific results can be used with confidence. In practice, however, this is not always the case and the value and validity of published research results are often debated. The root of such debates is often attributed to sector bias, but in actuality it is the uncertainty surrounding those results that prevent people in all sectors from trusting them wholly, too often paralyzing the decision making process. Uncertainty is inherent in all science; no research results will ever have 100% certainty. The root of individual and sector bias is discomfort with uncertainty and never being able to truly know which side of the confidence interval an environmental reality may actually lie for any given scenario. If the collective research community was able to minimize uncertainty to unmeasurable levels, sector bias would cease to exist. This presentation uses examples for various environmental models to demonstrate how individual and sector perspectives lie in different regions of the proverbial cone of uncertainty, and demonstrates that what may present as bias is often an affinity towards a particular location within uncertainty bounds. If individuals or sectors are all existing within the same space of uncertainty, then no one perspective is wrong. Rather than forcing agreement on where within an interval of uncertainty the "truth" lies, efforts of the collective research community should focus on identifying, reducing, and characterizing uncertainty. As the breadth of uncertainty decreases, so will the distance between perspectives of individuals and sectors within the regulatory community.

**425 Reducing bias through multi-sector collaborations**

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Bias in scientific research can be defined as the over-reporting of false-positive or false-negative results, which are those that cannot be replicated or turn out to be invalid when applied beyond the experimental construct. Thus, bias is a function of how research is conducted, interpreted and characterized. Multi-sector collaborations (MSCs) are proffered as a means of reducing bias, but their success depends upon the extent to which inherent impediments are recognized and addressed. Particularly within the realm of public and environmental health, contributors may have divergent views of the problem to be addressed and may disagree on the underlying principles of what science itself requires. Participation in MSCs can be motivated more by a desire to advance agendas important to particular sectors rather than to achieve solutions that provide general benefits. In some instances, maintaining the perception of being at odds with other stakeholder groups is highly valued, particularly when particular interest groups consider preservation of an issue or controversy more beneficial than problem-solving. The opposite occurs when stakeholders value a reputation as cooperative and conciliatory more highly than finding optimal solutions. These impediments can render "collaboration" mere "negotiation" or even "confrontation." The most critical factor in overcoming these impediments is the ability to recognize them and to confront them candidly. These must be addressed in order to determine whether a true collaborative effort is even possible. MSCs should begin with a clear problem formulation step that achieves the full consensus of all participants. There must also be forethought regarding the underlying principles involved and a plan for identifying incongruent views of the collaborators, e.g., what is meant by relevance and quality of data, what is the appropriate role of expert judgment, and whether scientific objectivity or precautionary values are appropriate for the endeavor. To decide whether a true "collaboration" is possible, it is critical to fully vet these principles and develop a clear statement regarding areas of consensus and disagreement, the motives for participation, potentially conflicting goals, and the types of outcomes that will benefit each sector. To the greatest extent possible, team approaches should be used in order to reduce the inherent tendency toward negation rather than collaboration.

**426 Citation trends in the ecotoxicology literature and what they tell us about possible biases**

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There seems to be a reticence to engage with the totality of the pesticide ecotoxicology literature, especially papers that report few or no effects or low risk to non-target organisms. We hypothesized that these studies would have fewer citations than studies that report significant effects or risk for the same compound, and this would be unrelated to the strength of the study, e.g., high quality studies with few or no effects would be cited less frequently than studies of lesser quality that reported effects. To investigate this, we examined a subset of literature around the herbicide atrazine and responses in fish, amphibians, and reptiles. We found that papers reporting an effect had significantly more citations per year than those that did not ($p < 0.05$). There was no significant relationship between the strength of the study and number of citations, but a general trend for weaker studies to have greater number of citations. The impact factor of journals was not positively correlated with the strength of the study methods, but studies that reported effects were published in journals with a greater mean impact factor than those that reported no effects ($p < 0.05$). This analysis reveals evidence of citation bias within the pesticide ecotoxicology literature, as well as bias by journals to publish studies that report effects, regardless of study quality. We have built on this initial work and are examining citation trends for articles published in the journal Environmental Toxicology and Chemistry (ET&C). Specifically, we focused on papers reporting effects of pesticides since ET&C's inaugural issue in 1982 through to 2012 and limited the analysis to the titles only

(>700 papers). A number of questions were addressed in addition to role of 'effect' or 'no effect' and findings will be presented. These included; does author affiliation influence citation rates (e.g., academic, industry, government), does the class of specific pesticide matter, and have trends changed through time? In conclusion, ecotoxicologists seem to have a problem with 'no effect', whether publishing, interpreting, or citing such findings. This bias towards seeing 'effects' everywhere has the potential to severely hinder our ability to identify real problems and work collaboratively to address these challenges. Recommendations to address this behaviour will be provided.

**427 The role of transparency in enabling reproducibility and reducing bias**

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In March 2018, the US Environmental Protection Agency (EPA) proposed a rule titled *Strengthening Transparency in Regulatory Science* (83 Fed. Reg. 18768). The title is misleading, however, because EPA proposes to restrict its increased focus on transparency to only dose-response data and models, to only final regulations, and to only *pivotal studies*, as narrowly defined in the proposed rule. Worldwide, scientists and science organizations have recognized the crucial role of transparency to the crux of the scientific enterprise: reproducibility. Any agency that promulgates environmental regulations should require that science considered as possibly key or pivotal to a regulatory purpose must meet a standard of transparency. The role of environmental agencies is to translate and distill research results into regulations, guidance, and policies that have significant impacts in the real world. It is therefore an obligation to ensure use of the best available science on any issue, for any important matter of policymaking. Whether in the "pure" or regulatory sciences, the principal reason for requiring transparency of scientific data is to enable reproducibility studies and validation. An inability to reproduce or validate a research result is often related to a bias in the original study. For an agency to require transparency but ignore findings of irreproducibility defeats the purpose. Thus it is important to develop mechanisms of scientific review that incorporates risk of bias and validation studies. Governments belonging to the Organization for Economic Cooperation and Development require that scientific testing data submitted by industry as the basis for product registrations, hazard evaluations, or other purposes comply with *Good Laboratory Practices* (GLP). The reason for the requirement is to ensure that the Agency's evaluations are based on *data of sufficient quality, rigor and reproducibility*. Recognizing that not all provisions specified by formal GLP programs can be applied to all studies, the concept of the *spirit of GLP* is often adopted by regulatory agencies and study sponsors. GLP and the spirit of GLP should be adopted by environmental agencies as a requirement for all science considered as a possible basis for regulation, guidance, or policy. Doing so would be a step towards reducing bias and enabling replication.

**428 Assessment of chemical risk employing field investigations to address hazard and exposure: Identification and management of bias**

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Emerging contaminants and other stressors that receive heightened public attention often attract investigation because there are increased pressures from management or regulatory agencies to assess the risk, they offer the potential for funding opportunities, or they offer opportunities to address issues of interest to the scientific community. In developing field or laboratory assessments, investigators need to be careful to develop their assessment strategy to not adversely or unknowingly bias the results or interpretation. Determining ecological risk often requires assessing impacts to organisms using field exposures and linking them to laboratory derived toxicological results (effects). For emerging contaminants, information is often limited or rapidly evolving, making it difficult to characterize background concentrations, and the environmental fate and transport, toxicity, and bioaccumulation/biomagnification are often not well understood; therefore, it is possible that environmental samples may not adequately describe the natural variability or range of concentrations to which receptors may be exposed. There is also often pressure to complete assessments of emerging contaminants to understand the ecological risk; however, this pressure may also contribute to bias, when our need for large-scale answers means overlooking the limitations of key underpinning data. Incomplete understanding of the toxicity of the emerging contaminant may also be a source of bias regarding effects. This limited information may potentially increase the chances that the endpoints, modes of exposure, or species evaluated are irrelevant to addressing the overall risk. Using case studies, this presentation will provide examples where the results from field or laboratory studies may be misleading or bias the interpretation of adverse effect beyond what the data can support or what the study was designed to assess. Recommendations for strategies that can be used in managing bias when it is identified will also be provided.

**429 The interaction of different forms of bias in academic research and suggestions for mitigation**

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In academic research, numerous forms of bias exist. Research bias can arise because of the structure of academic institution and granting agencies, and their associated reward structures. The scientific research conducted could be biased because of a study design that emphasizes novel results. The particular question the research addresses could be biased because of the reliance and acceptance of significant findings. Other layers of implicit bias exist that include social and gender biases that affect the type and quantity of research generated. In this presentation I will discuss these biases and how they interact, with examples from academic research institutions, and I will address how they affect ecotoxicological research. I will also discuss potential mechanisms to mitigate these biases so that we can come up with solutions to pressing ecotoxicological questions more efficiently and equitably.

## Environment Exposure to Microplastics and Affiliated Toxic Chemicals

**430 Polycyclic aromatic hydrocarbons affiliated with microplastics in surface waters of Bohai and Huanghai Seas, China**

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Microplastics (MPs) sized between 0.33 and 5 mm were collected using Manta trawls from ten surface seawater sites in Bohai and Huanghai Seas, China. A total of 1,024 (Bohai Sea) and 132 (Huanghai Sea) MP pieces were classified, including polystyrene foams, polyethylene films and lines, and other plastic pellets, with concentrations of MPs ranging from 3 to 162 particles per 100 $m^3$ (0.012 to 2.96 mg $m^{-3}$). Apretreatment of MPs with 30% $H_2O_2$ in water did not significantly lower polycyclic aromatic hydrocarbon (PAH) concentrations on MPs compared to no $H_2O_2$ pretreatment. Measurements of PAHs carried on the collected MPs indicated that the concentrations of the sum of 16 PAHs were in the range of 3,400-119,000 ng $g^{-1}$. The sources of PAHs in Bohai and Huanghai Seas were highly similar, with petroleum and gasoline probably as the dominant sources. The present study shows the relative importance of MPs in regards to chemical transport in the marine environment. The combination of high concentrations of PAHs affiliated with MPs and the increasing magnitude of plastic pollution in the world's oceans demonstrates the considerable importance of MPs to the fate of PAHs in marine environments.

**431 The influence of particle charge and eco-corona on the entry of nanoplastic into a freshwater food web**

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Plastic particles are an abundant contaminant in the aquatic environments. Microplastic particles (< 5 mm) are of concern, as they have been demonstrated to enter food webs. However, little is known about their smaller counter parts, nanoplastics (< 100 nm, NP), largely because their quantification in the environment remains a challenge. A small number of studies have quantified nanoplastic uptake via waterborne exposures within aquatic organisms, but not enough is known about how NPs may enter aquatic-food-webs, and what might modulate this entry route. In this study, the entry of nanoplastic into the base of a food web, was quantitively tracked, for the first time. Nanoplastic sorption to *Chlorella vulgaris* Beij. (green algae) and the subsequent dietary transfer of nanoplastic via the algae to *Daphnia magna* (water flea) was studied. The modulating role of nanoplastic surface charge, and eco-corona formation (where particles were coated with humic acids or proteins to simulate the effects of environmental alterations in their surface chemistry), was addressed. Quantification of NP tissue burdens (LOD: 0.08 µg/mg) was enabled using a novel fluorescence bioassay in a high-throughput microplate format, supplemented with airyscan-confocal imaging, to trace the particle interactions with algae-cells and the dietary transfer to *Daphniids*. *Daphnia magna* whole-tissue oxidative stress response was also investigated, as an indicator of NP systemic bioavailability, following dietary transfer. Particle charge had a significant effect on the NP algal-cell-particle sorption, with positive particles showing higher algae-sorption and dietary transfer, than negative or neutral particles. Eco-corona formation had no significant effects under the conditions tested. Bio-imaging confirmed NP-algae localization in the *D. magna* gut, and no alterations in oxidative stress responses were found. In conclusion, we successfully tracked the entry of nanoplastics into the base of a food web, where we found that particle charge will significantly affect the amount of NPs that enter a freshwater food chain. This finding supports that the physico-chemistry of the particle in the environment is an important factor for determining NPs entry into a food web.

**432 Ingestion, bioaccumulation and depuration of nano- and microplastic particles by marine bivalves**

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Plastics debris are introduced into the oceans, through industrial production and as anthropogenic waste. Larger plastics breakdown into nanoplastics (NP) and microplastics (MP) via weathering. Benthic animals, such as suspension-feeding bivalves, are exposed to NP and MP pollutants in coastal waters. Studies have shown that NP and MP negatively affect marine animals on an organ and cellular level. Despite the potential for exposure and toxicological effects, the uptake and accumulation of NP and MP by bivalves is largely unexplored. This study examined the ingestion, egestion, and bioaccumulation of fluorescent polystyrene NP and MP by the blue mussel (*Mytilus edulis*). NP and MP were aged in seawater for 3 days prior to exposure experiments. Mussels were exposed to a 0.1 mg/L/hr concentration of NP or MP for two weeks and then allowed to depurate for one week in filtered seawater. Mussels were fed a standard microalgal diet throughout the 3-week experiment. Whole animals were frozen for later analysis of NP or MP concentrations at the end of each week and feces were collected daily for all 3 weeks and frozen. Tissue and feces samples were analyzed via a scanning fluorescence spectrophotometer for plastic quantification. The concentration of NP in tissue samples did not differ significantly between 7 days and 14 days of exposure, nor did the concentration of MP. After the exposure period, 38.5% of NP and 63.3% of MP remaining in the mussels were egested on the first day of depuration. Feces samples contained higher concentrations of MP during exposure and tissue samples contained higher concentrations of NP during exposure. These results imply that plastic particles are transported in and out of mussels quickly. This study will be used to develop biokinetic models of NP uptake and bioaccumulation in shellfish and help elucidate the potential for these materials to be passed to higher trophic levels including humans.

**433 Sorption of Antimicrobials, Triclosan and Triclocarban, to Polyethylene and Polypropylene Microplastics**

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Microplastics enter rivers and estuaries through stormwater runoff and municipal wastewater effluent, where they eventually travel to the ocean. Recent research has suggested that billions of microplastics per day may enter U.S. waterways from municipal wastewater. Organic contaminants are known to sorb to plastics at concentrations magnitudes higher than the surrounding water. However, there is relatively little research about the behavior of compounds in pharmaceutical and personal care products, which are concentrated in wastewater. Most work has focused on compounds with only nonpolar moieties, such as polyaromatic hydrocarbons and polychlorinated biphenyls. Therefore, this study examines the sorption behavior of the antimicrobial agents triclosan and triclocarban to microplastics made of high-density polyethylene and polypropylene. In addition, efforts were made to create a standard protocol for fabricating microplastics at different particle diameters, characterizing particle surface area, and measuring the sorption behavior of slightly-polar organic compounds on microplastics. Results will be presented that will show the differences in sorption between particles of different diameters (i.e., surface area) and polymer types. The standardization of these methods will increase the robustness of future research, so that data may eventually inform wastewater policy.

**434 Microplastic is a Vehicle for Pharmaceutical-Transforming Microorganisms**

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Microplastic is an anthropogenic contaminant that is widely distributed in the environment. As microplastic travels through aquatic systems it will interact with and may bind other environmental contaminants, as well as microorganisms. We propose that microplastic is a vehicle that carries microorganisms and pharmaceutical contaminants, which serve as a source of carbon and energy for microbes, from wastewater treatment into the environment. To study the interaction between microorganisms, microplastic, and pharmaceuticals, we established methanogenic enrichment cultures using anaerobic digester sludge as inoculum and delivered naproxen as the sole carbon source by coating microplastic beads with it. Naproxen is a nonsteroidal anti-inflammatory drug that is frequently detected in wastewater-impacted urban environments. HPLC analysis of primary enrichment cultures showed naproxen loss and accumulation of a metabolite in the aqueous media. Once naproxen transformation was complete, colonized microplastic and planktonic cells from the bulk medium were separated and independently used to inoculate fresh medium containing neat naproxen. Naproxen was again completely transformed in anaerobic microcosms inoculated with transferred microplastic, but no loss was observed with transfers of planktonic cells. Next-generation DNA sequencing showed differences between the two microcosms with respect to the microbial community structure and presence of specific functional genes. Our results demonstrate that microplastic provides a habitat for microorganisms that allows them to be in close contact with each other and with sorbed contaminants that serve as a source of carbon. This creates the potential for the environmental transport of pharmaceutical-degrading microbes on the microplastic surface.

**435 Trophic transfer of microplastics and an associated legacy pollutant from microzooplankton to their predators**

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Microplastics, which pose an environmental hazard because they are readily ingested and accumulated by aquatic organisms, are of increasing global concern in the aquatic environment. The plastic attracts and dissolves lipophilic compounds, such as the legacy pollutant DDT, that may leach into the tissues of organisms upon ingestion. This study used larval inland silversides, *Menidia beryllina* and the tintinnid ciliate, *Favella* spp as a predator-prey model relevant to US estuaries, in order to examine trophic transfer of microplastics. LDPE microspheres (10-20µm) treated with the insecticide DDT were used to determine whether the presence of a plastic-associated pollutant can affect plastic ingestion by larval fish and their prey. After allowing ciliates to feed upon either contaminated or virgin microspheres, larval fish (5 dph) were permitted to transiently prey upon them. After the 2 h feeding period, larvae allowed to transiently prey upon plastic-laden ciliates were found to contain up to 320 particles larvae$^{-1}$. Larvae in direct ingestion groups ingested significantly fewer microspheres than those in trophic transfer groups. This suggests trophic transfer from prey could be a significant source of microplastic exposure. Larval silversides ingested significantly more ciliates fed DDT- treated microplastics. Retention time of microplastics in the gut of larval silversides was also investigated. The excretion rate of microplastics integrated over the entire sampling period (2.5h – 72h after feeding) was 0.15 particles h$^{-1}$, with most particles being excreted within 24 h. Fish reared for a further 16 d following a 2 h microplastic feeding had a lower weight than individuals that were not exposed to microplastics. This is the first study to demonstrate the trophic transfer of microplastics in an estuarine predator-prey model. Our results indicate microzooplankton can be vectors for the transfer of microplastics to higher trophic level organisms in estuarine systems and that a brief period of microplastic ingestion can impact growth and development of larval forage fish.

**436 Analytical and in vitro Estimates of Estrogenicity from Simulated Digests of Plastic Items**

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Since their mass production in the 1940's, plastic has become an indispensable part of modern life. Poor management of plastic waste results in the leaking of approximately 8.0 million metric tons of plastic into the world's oceans every year. Over 206 species, including 119 bird species and 41 fish species have been documented to ingest plastic, likely confusing it for prey. Once ingested, plastic may release endocrine-disrupting compounds which may impact development, reproduction and success at the individual level and declining trajectory at the population level. We selected 16 plastic items that are most commonly found ingested by marine animals. We then "digested" these plastic items in fish and seabird laboratory gut mimic models using the digestive enzyme pepsin at pH 2 and shook them for 16 hours at either 28° C (in saltwater) for fish or 40° C (in freshwater) for seabirds. Plasticizer content desorbed into the gut mimic liquid was quantified using solid-phase extraction followed by ultra-high-performance liquid chromatography/tandem mass spectrometry. Polymer types were identified using Fourier-transform infrared spectroscopy. In vitro estrogenicity was assessed using a recombinant cell line. Out of 12 plasticizers analyzed, 4-*tert*-octylphenol (OP), bisphenol A (BPA), bisphenol S, butylbenzyl phthalate and bis(2-ethylhexyl) phthalate (DEHP) concentrations were significantly increased due to digestive gut conditions. Desorption of estrogenic plasticizers from plastic items was significantly higher in the seabird gut mimic than the fish gut mimic. Only micro-sized plastic items (< 5mm) had higher in vitro estrogenicity due to gut mimic conditions. Of the plastic items tested, expanded polystyrene, shopping bag fragments and polypropylene string had both the highest analytically- and in vitro -determinedestradiol equivalency values (EEQ) in both seabird and fish gut mimic conditions (range: 2.8-9.6 ng/g). Enhanced desorption of OP, BPA and DEHP accounted for the higher in vitro estrogenicity. These results demonstrate the need for further research into the endocrine-disrupting effects of plastics once ingested.

**437 Interaction and immunotoxic effects of microplastics in the estuarine worm *Hediste diversicolor* in environmentally relevant conditions**

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Since the early 1970s, presence of floating plastic has been reported in marine waters with great accumulation in gyres. In recent years, the presence of plastic debris < 5mm called microplastics (MPs) which result mainly from macroplastic's fragmentation has also been reported in aquatic ecosystems. Several studies have reported that these MPs are persistent and their accumulation has been observed in various aquatic species. The majority of studies have focused on marine species, but much less with continental and estuarine biota. The present study investigates the interaction and the effects of microbeads and a mixture of two types of MPs (polyethylene and polypropylene), frequently found in natural environments, towards the ragworm *Hediste diversicolor* to determine their accumulation in animals exposed through the water phase or sediment. First, to get a better understanding of the interactions between MPs and the ragworm, organisms were exposed to fluorescent microbeads and the tissues were observed under a fluorescent microscope to follow the path of MPs. Then, two concentrations of exposure were selected corresponding to medium and heavily contaminated areas reported for water phase (10 and 100 µg/L) and sediment (10 and 50 mg/Kg) and organisms were exposed for 96h or 10 days following the protocol of the American Society for Testing and Materials (ATSM, 2013). To study the potential toxic effect of MPs, immune parameters have been selected since they are involved in many defense mechanisms against external agents. An average number of MP/worm ranging from 0 to 2.5 and from 1 to 36 was identified in animals exposed to the lowest and the highest concentration of MPs respectively, through water exposure. However, the majority of MPs seem to be located into the worms skin mucus. In worms exposed through sediment, less than 1 particle/worm was found and a higher number of particles were identified in depurated sediment. First results of immunotoxic impact indicates that MPs exposure induced a decrease in coelomocytes viability and in phenoloxydase activity, but no alteration of phagocytic activity was measured. This study brings new results on the potential accumulation and immunotoxic effects of MPs, in environmentally relevant conditions, for the ragworm *H. diversicolor* who plays a key role in the estuarine ecosystem.

## Assessing Contaminant Effects on Early Life Stages of Marine Organisms

**438 Age-dependent partitioning of polychlorinated biphenyls among the brain, liver, kidney and muscle of a coastal fish predator**

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Polychlorinated biphenyls (PCBs) are globally- distributed pollutants that bind to adipose tissues found throughout the bodies of multicellular organisms and, at high levels, can induce detrimental health effects. As organisms age from juveniles to reproductive adults, the relative mass of adipose tissue in the brain, liver, muscles and other organs often shifts.

However, it is unclear how these ontogenetic changes may also affect PCB concentrations in different organs, because most studies have measured PCBs in a single organ, whole body composites, or organisms of a similar age. To address this gap, we quantified the lipid content and PCB concentrations of brain, kidney, liver, and muscle samples taken from 15 red drum (*Sciaenops ocellatus*), a large, long-lived predatory fish common to southeastern and Gulf US coastal waters. We discovered that lipid normalized PCB concentrations increase in all four organs as fish age, however their rate of increase was significantly faster in liver, brain and muscle tissues than in the kidney. When multiplied by the mass of each organ, we found that while the liver contributes most to an individual's total body burden for young red drum, muscle tissues contribute most for older individuals when lipid normalized. These findings highlight the importance of ontogenetic shifts in influencing rates and patterns of PCB accumulation.

**439 Impacts of microplastic fibers on Pacific mole crab (*Emerita analoga*) development and mortality**

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Microplastic is an emerging pollutant in marine and coastal ecosystems. Millions of tons of plastics are added into these systems annually and are of particular concern due to their persistence in the environment, propensity to attract other pollutants, their toxicity and tendency to degrade into microplastics (particles or fibers < 5mm) making them easily ingestible. Sandy beaches are consistently exposed to plastic debris accumulation from wave action, near shore currents as well as effluent from waste water treatment plants, exposing the infauna to persistent plastic pollution. Pacific mole crabs (*Emerita analoga*),found in the sandy beaches along the Pacific coast, are filter feeders that have been shown to ingest microplastic in previous studies. To assess the impact of polyethylene fibers on the reproductive development of Pacific mole crabs, adult females were exposed to environmentally relevant concentrations of polyethylene fibers (< 1mm) for 2 months during a reproductive cycle. Effects were investigated on offspring development. Our study shows that the exposure and ingestion of polyethylene microplastic debris at environmentally relevant concentrations alters development in Pacific mole crabs and warrants further research.

**440 An assessment of directly ingested and trophically transferred microplastics in larval and juvenile black sea bass (*Centropristis striata*)**

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Plastic pollution, primarily in the form of microplastics, is the most abundant and persistent form of marine debris. Coastal and estuarine waters are especially susceptible to microplastic pollution due to their proximity to terrestrial inputs and tidal processes that provide favorable conditions for accumulation. Estuaries are critical habitats for early life stage development of commercially important fish species, including the black sea bass (*Centropristis striata*). Trophic transfer of microplastics and associated pollutants is documented, but the impact is still largely unknown. To evaluate the role and effects of microplastic ingestion in this commercially important species, larval and juvenile *C. striata* were exposed to three concentrations (1,000,000 particles $L^{-1}$, 100,000 particles $L^{-1}$, and 10,000 particles $L^{-1}$) of virgin and contaminated 10-20 mm low-density polyethylene (LDPE) microspheres directly in the water and via trophic transfer from prey. Larval fish were also directly exposed to LDPE microfibers, and juvenile fish were exposed to fibers directly and via prey. For trophic transfer treatments, juvenile sea bass (60 days post hatch) were fed larval silversides (*Menidia beryllina*), and larval sea bass were fed single-celled ciliates (*Favella* spp.), both containing plastics. The contaminated microspheres were treated with either 1.9 mg/g of phenanthrene, a polycyclic aromatic hydrocarbon commonly detected on plastics or 12 mg/g of 2,4-di-tert-butylphenol, a hindered phenol added to plastics to prevent thermal degradation during processing, both at environmentally relevant concentrations. Microsphere counts are being obtained for each larva to determine consumption across all treatments after a single 2-h exposure, and RNA-Seq will be conducted to measure gene expression. Juveniles were exposed to microplastics over a 4-d period, and sublethal effects, including oxygen consumption and immune response were assessed at test termination. Whole juvenile fish were also preserved for chemical analysis to determine if the pollutants leach into the body tissues of the fish. We hypothesize that cultured larval and juvenile *C. striata* will be negatively affected by microplastic exposure, chemicals leach into tissues, and microplastics are possibly transferred in greater numbers from trophic transfer than direct ingestion. Results of this study will lend guidance to management of the fishery and inform potential concerns regarding human consumption.

**441 Biomarker Responses of Coral Larvae**

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Coral reef ecosystems are among the most biologically diverse and productive ecosystems on the planet, and provide habitat for a myriad of fish and invertebrates. Therefore, coral reef biologists and conservationists are actively engaged in research for finding ways to improve the recruitment and sustainability of coral species. One common approach has been to use asexual propagation techniques in which broken pieces of coral are glued to a calcium carbonate substrate and placed into the field to restore coral reef ecosystems. Researchers at CARMABI (Caribbean Research and Management of Biodiversity (CARMABI) research station in Curaçao have also recently been working with SECOR (Sexual Coral Reproduction), a program which focuses on rearing larvae in the laboratory to improve settlement and recruitment of coral larvae, and then planting the young coral colonies for repopulating coral reefs in situ. There are concerns about the best habitat for restoration and potential anthropogenic effects of pollutants, including sunscreens. We conducted biomarker studies, especially those related to oxidative stress (the antioxidant glutathione and lipid peroxidation as an indicator of oxidative damage) in adults and larvae of three species of Caribbean corals (*Diploria labyrinthiformis, Tubastrea coccinia, Agaricia humilis*). Very little information has been published regarding biomarkers of antioxidant levels in coral species, and baseline levels of glutathione can serve as a potential indicator of differential sensitivity. Overall larvae had significantly lower gluathione levels than adults, suggesting that larvae may be especially sensitive to algal or environmental stressors. Biomarker studies were also conducted with larvae that were exposed to various benthic algae that could affect settlement and metamorphosis success, and there was evidence of potential allelochemical production by some of the algal species that resulted in oxidative stress. We also worked with larvae of one species of Hawaiian coral, *Montipora capitate*, and conducted preliminary experiments in which larvae were exposed to different sunscreens (including Zn, Ti, and oxybenzone formulations). Understanding factors that can affect recruitment and settlement of coral larvae and the development of sensitive bioassays of larval health are critical aspects of reef preservation and restoration.

**442 Developmental and reproductive effects in grass shrimp (Palaemonetes pugio) following acute larval exposures to thin oil sheens and ultraviolet light**

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One question after an oil spill is the toxicity of thin oil sheens to early life stages of aquatic species and if that toxicity can be magnified by interaction of hydrocarbon compounds with ultraviolet (UV) light. Many early life stages congregate at the surface or in the upper mixing layer making them prone to UV light exposure and thin sheens of oil at the surface. Laboratory testing was used to assess UV-oil sheen interactions with grass shrimp (*Palaemonetes pugio*), a common estuarine species. Newly hatched grass shrimp larvae were exposed to a 1 µm thick fresh oil sheen for 24 h with or without a 6-h pulse of a UV light. Grass shrimp were then moved to clean seawater and non-UV conditions to measure development, growth, and reproductive fitness. Minimal toxicity was observed after the initial exposure but larval life cycle development was significantly delayed in shrimp exposed to the UV enhanced slick. After reaching sexual maturity, shrimp were paired to evaluate effects on reproduction. Shrimp initially exposed to the UV enhanced slick as larvae had a significant reduction in fecundity compared to controls. This demonstrates the importance of examining the interactions between UV light and oil since negative effects to aquatic organisms may be underestimated if based on standard laboratory testing with fluorescent lighting. Acute exposures of early life stages to thin oil sheens and UV light may lead to long-term impacts to individuals and ultimately to grass shrimp populations.

**443 Lipid, growth and 1st year survival impacts in Polar cod (*Boreogadus saida*) following embryonic oil exposure to Alaskan and Norwegian oil**

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Recent evidence suggests that embryonic oil exposure impacts the lipid metabolism and cardiocirculatory function in marine fish. Polar cod *Boreogadus saida* may be particularly vulnerable to oil because they are an energy-rich forage species that depends on stored lipids to successfully overwinter in the Arctic. In 2017, we tested this hypothesis by exposing embryos to physically dispersed microdroplets of Alaskan oil for 3 days during cardiac organogenesis, followed by a 4-day washout period and transfer to clean water. Total polycyclic aromatic hydrocarbon dose concentrations in sea water were $0.9 \pm 0.3$, $3.3 \pm 0.4$, and $15 \pm 5$ µg/L and $63 \pm 11$, $505 \pm 105$, and $1170 \pm 170$ ng/g wet weight in embryos, respectively. Embryos were assessed for oil-induced cardiotoxicity immediately after exposure (28 dpf) and at hatch (42 dpf). Biometrics and lipid composition were determined at several points up to ~150 days post-hatch. For the 15 µg/L and 3.3 µg/L exposure concentrations, either all or most of the hatched larvae had severe craniofacial malformations. Large numbers of larvae with a normal morphology from the 0.9 µg/L exposure showed significant long-term growth impairment. There was a dose-dependent increase in triacylglycerols (TAG) and free fatty acids (FFA) in yolk sac larvae that was not present in the egg-phase but that persisted through first feeding. However, in surviving juveniles (0.9 µg/L exposure), TAG levels were reduced after flexion relative to controls. In 2018, a similar experiment was conducted using Norwegian oil in order to determine the lowest effective exposure concentration, and track bioenergetic impacts beyond 150 days post-exposure. Preliminary findings suggest that 0.3 µg/L exposure concentrations do not statistically impact growth phenotypes, but tissue analyses and respiration measures are ongoing. Collectively, these data suggest that storage and fuel lipids (TAG and FFA) are underutilized or inappropriately synthesized (or both) in exposed first-feeding larvae, while these lipids are burned excessively in juveniles rather than accumulating for overwintering. This supports an exposure and injury model in which embryonic contact with oil leads to irreversible lipid-bioenergetic deficits which in turn could underlie poor larval growth, and consequently, reduced recruitment due to high overwintering juvenile mortality.

**444 The oxidative stress response of developing zebrafish embryos exposed to hypoxia and a complex mixture of polycyclic aromatic hydrocarbons**

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Estuarine and riverine ecosystems are often important nursery habitats for aquatic species. These are highly dynamic systems, often plagued with increasingly prevalent environmental stressors, such as hypoxia. They also act as sinks for anthropogenic contaminants, including polycyclic aromatic hydrocarbons (PAHs). Embryonic co-exposures to PAHs and hypoxia have been found to result in compounding toxicity in multiple fish species, although the mechanisms by which this occurs are not fully understood. Based on evidence that both hypoxia and PAH exposures induce oxidative stress in developing organisms, we hypothesized that this mechanism could play a role in the interactive toxicity seen after co-exposures. To test this, we exposed zebrafish embryos to non-teratogenic doses of a real-world complex PAH mixture with and without hypoxia beginning at 6 hpf. After 24 hours of exposure, embryos were removed from exposure conditions and were either used immediately for experimentation or placed into clean "recovery" conditions for either 1, 5, or 18 hours before sampling. Our endpoints target several facets of the oxidative stress response: pro-oxidant formation, antioxidant response, oxidative damage, and cellular signaling. We measured changes in gene expression and enzyme activity for several antioxidants including glutathione peroxidase, glutathione reductase, and catalase, utilized the *hsp70* and *epre* transgenic zebrafish lines to monitor alterations in Nrf2 signaling activity and measured non-specific ROS formation with the fluorescent probe DCF. Preliminary data suggests that PAH exposures alone result in significant inductions of glutathione reductase enzyme activity across several time points of recovery, although hypoxia exposures do not. PAH exposures with and without hypoxia also appear to induce Nrf2 activity in *epre* transgenic zebrafish. Hypoxia exposures caused a slight, but not statistically significant, increase in Nrf2 signaling in *hsp70* transgenic zebrafish. Thus far, there do not appear to be interactive effects of hypoxia and PAHs on the oxidative stress response of developing zebrafish embryos immediately after removal from exposures. Future work will focus on periods of re-oxygenation, as these are more likely to induce oxidative stress in fish species.

**445 Development of a new regulatory test method for the marine copepod *Acartia tonsa***

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A 10 year review of the Canadian Metal Mining Effluent Regulations (MMER) has recently been completed and has identified the need for a fish and invertebrate test method suitable for assessing the acute lethality of saline effluent discharged into marine or brackish environments. The freshwater invertebrate *Daphnia magna*, currently used to monitor the acute lethality of mine effluents in the MMER, is inappropriate for toxicity testing of saline effluents to be discharged to marine or estuarine environments as *D. magna* is intolerant of salinities in excess of 4 ‰. To fill this gap in regulatory testing, Environment and Climate Change Canada launched an initiative to develop a Reference Method using the marine copepod, *Acartia tonsa,* to measure the acute lethality of saline effluents. The proposed test method will be based on a 48 hr exposure starting with the egg stage, whereby newly laid eggs are obtained from breeding male and female copepods. The test endpoint will be survival, which will be expressed as hatching and mobility. Research and method development has been conducted over the last 2 years, and included: determination of the conditions needed to establish in-house cultures of *Rhodomonas salina* (an algal food source) and *A. tonsa*; reference toxicant testing with nickel chloride ($NiCl_2$); development of test methodology (including test endpoints and validity criteria) and; management of two rounds of an inter-laboratory study involving definitive tests with $NiCl_2$ and phenol to validate the proposed test method. One of the key aspects of the inter-laboratory study will be to use the data acquired to establish reasonable acceptability criteria for control organism survival (with consideration given to both % egg hatching success and % mobility). This presentation will provide an overview of some of the research conducted to date, including lessons learned and the results of both rounds of inter-laboratory testing (involving six labs across Canada).

## Exposure to Crude Oils: Linking Laboratory Endpoints to Field Observations

**446 Chemical and physical characteristics of oil in aquatic environments: Comparison of laboratory-prepared exposures to field observations**

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As oil moves through marine and freshwater environments, a variety of physical and chemical processes can occur. These processes can impact the chemical composition and physical condition of the oil, where the oil goes, and ultimately how, and to what, aquatic organisms are exposed. Linking the potential impacts of oil spilled in the environment to laboratory-based endpoints requires understanding how oil behaves under different field conditions and relating that to the chemical and physical characteristics of the oil contaminants for different toxicity testing exposure scenarios. Through different oil spill research efforts, we have conducted extensive characterization of different toxicity testing exposure scenarios including three commonly used water-accommodated fractions (WAFs) and different oil slick preparations. We have also characterized the chemical composition and physical condition of oil slicks and dispersed oil within aquatic environments in the field. In this presentation, we will present results from our characterization of WAF and slick preparations for laboratory-based toxicity tests and discuss how they relate to our observations in the field.

**447 Contaminated Sediments are the Source of Surface Sheens in the Northern Gulf of Mexico Around the Toppled Taylor Energy Platform**

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In 2004, a production platform owned by Taylor Energy in the Mississippi Block 20 of the northern Gulf of Mexico was toppled by a mudslide during Hurricane Ivan. Fourteen years later, surface sheens continue at the site, but the source (s) and location (s) have not been fully constrained. Past activities at the site that disturbed the bottom sediments led to some of the largest relative estimates of daily releases. Hence, moving forward it is necessary to gauge the relative sources with an understanding that actions at the seafloor increase load to the environment above the sediment. The situation at MC20 differs from the norm established by many spills in that approximately 20 different wells (endmembers or "source" oils) were released into local bottom sediments, the water column, and the sea surface. Sheens are still observed even after well interventions were completed in 2011. Potential source(s) and location(s) on the seafloor were identified after two major field studies in 2012/2013. Two distinct zones of heavily contaminated sediment exist on the seafloor at the excavated former well-bay area and near the toppled jacket, approximately 600 feet downslope. Other locations sampled at the same time, even within six meters from these heavily contaminated sites, had much lower concentrations of total petroleum hydrocarbons and distinct chemical "fingerprints". In 2017, over a six-week period, we collected ~60 surface sheen on 10 separate days that were also geospatially and geophysically time-stamped (ocean currents and sonar). We analyzed these samples for biomarker ratios and used cluster analysis to compare them to our historical database and each 2017 sample. Overall, the 2017 surface sheens are heterogeneous, do not appear to be from a single oil, and have the greatest similarity to samples from the highly contaminated chemically heterogeneous sediments near the toppled jacket. We argue that surface sheens are from remnant (released prior to well interventions) oil that rises out of the contaminated sediments and the varying locations of surfacing oil droplets causing the sheens is driven by currents. In addition, an erosion pit is located at the main release area. A mass-balance-based approach further supports this finding and reveals that a leaking well is not supporting the sheen at all or below a detectable level. This information is critical for evaluating potential mitigation actions including natural attenuation.

**448 Using in situ bioassays to monitor freshwater post-spill conditions over 3 years in Blacktail Creek, North Dakota**

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In situ and site water bioassays are an important tool to directly assess the ability of resident species to survive in post-spill environments. These experiments, while potentially labor intensive, are relatively short (96 h) and require minimal technology to perform. While this seems to be an uncomplicated approach, the interpretation of results in an uncontrolled field situation can quickly become more complicated. Determinations of cause and effect for observed mortalities are improved when in situ bioassay data are coupled with extensive organic and inorganic post-spill geochemistry data from identical sites at similar times. We monitored the survival of fathead minnow (a resident species), in Blacktail Creek, Montana at low flow for three years 2015 – 2017 following a 2014 wastewater-pipeline rupture and oil spill. During the first two years we performed in situ bioassays and during 2017, we used site water collected from multiple sites to conduct 96 h laboratory experiments. Four experimental sites ranged from 0.9 km upstream of the spill to 22.9 km downstream from the spill. Extensive mortality was documented at only the site 7.2 km downstream from the spill during 2015. Geochemistry data collected on Day 0 defined a signature likely from the spill products at the same site including elevated trace hydrocarbons, and a Sr isotope ratio reflective of the pipeline fluid. An oil-sheen was observed at the 7.2 km site at 72 h and two dead resident fish were also observed at this site only. At 96 h, 2.5% survival was observed in the experimental chambers at the 7.2 km site. Reduced temperatures and increased Cl (up to 568 mg/L) and $HCO_3$ (up to 727 mg $CaCO_3$/L) concentrations suggest a pulsed upwelling of groundwater into the stream at the time that mortalities were observed. Chemistry data also suggest that the deaths were most likely attributable to elevated ammonia (calculated from measured pH and ammonium concentrations of 3.4 mg N/L) released from groundwater upwelling apparently connected to the spill. Significant mortalities, oil sheens, and elevated ammonia were not documented during 2016 or 2017. While a chemical signature of the spill was still possibly present, it was less than in previous years. The question remains about whether the 96 h in situ or site water experiments conducted annually are sufficiently timely to observe any episodic ammonia fluxes into the river, or aquatic-health impacts, if they are still occurring.

**449 A laboratory based evaluation of biomarker selection used to monitor post-spill light crude oil exposure in fish**

*J. Baker, M. Gagnon, Curtin University / School of Molecular and Life Sciences*

One of Australia's worst oil spills occurred in August 2009, following a well blow out on a drill platform located 260 km off the northwest coast of Australia. An estimated 23.5 million L of light crude oil and condensate were spilled in to the offshore marine environment over 74 days before the oil release was successfully capped. Scientific monitoring of crude oil exposure in fish began immediately following the cessation of the oil release and were undertaken for 2 years post-spill. Research focussed on the use of liver detoxification enzymes (through ethoxyresorufin-*O*-deethylase activity of hepatic microsomes, EROD activity), biliary polycyclic aromatic hydrocarbon (PAH) metabolites, sorbitol dehydrogenase (SDH activity) and DNA damage (8-oxo-dG quantification) as biochemical markers to monitor crude oil exposure in captured fish. Results showed limited differences between oil impacted and non-impacted sites at any stage post-spill. Questions have therefore been raised regarding the suitability of these biomarkers for monitoring exposure to light crude oil, of which is compositionally very different to that of a heavy crude oil. A lack of higher molecular weight PAHs in light Western Australian crude oils, which are known to be responsible for inducing a response in many of the selected biomarkers, may be the reason for the lack of differences in the post-spill monitoring results. The current research aimed to test the biomarkers used in the aforementioned oil spill in a controlled laboratory setting. Fish (*Lates calcarifer*) were exposed to the water accommodated fraction (WAF) of light crude oil for a period of 7 & 14 days, with additional fish depurated for another 7 & 14 days, followed by analysis of the biomarkers used during the oil spill monitoring program. Results showed no response for EROD activity, SDH activity or DNA damage for any time period or concentration levels. PAH bile metabolites were a sensitive biomarker of exposure with a clear dose and time response. Findings highlight the need for the development of a range of sensitive biomarkers in fish, suitable for light crude oil exposure with many 'traditional' monitoring tools not the most appropriate selection for this type of exposure. The employment of these traditional biomarkers in a light crude oil spill scenario may lead to false impressions on the severity of impacts on the marine environment.

**450 Comparing transcriptional profiles of lab exposed and field collected flounder following the Deepwater Horizon incident**

*R.J. Griffitt, University of Southern Mississippi / Coastal Sciences; D. Wetzel, Mote Marine Laboratory / ELF; R. Takeshita, National Marine Mammal Foundation*

The use of large scale whole genome or transcriptome profiling techniques to assess subtle effects of contaminant exposure in aquatic or marine organisms has become standard. In the aftermath of the 2010 Deepwater Horizon oil spill, many research groups used microarrays or RNAseq to identify genes or pathways that were affected by oil exposure, research which lead to many valuable insights about the interaction between organisms and exposure history. Often these are based on controlled laboratory exposures, which allow researchers to tightly constrain the experimental setup, and to ascribe the resulting transcriptional changes to the exposure with some degree of history. Transcriptional profiling using field collected organisms is significantly more challenging to perform and interpret with any degree of certainty, due to the lack of ability to control the dose or environmental factors that may affect the response. When possible, comparing the results obtained from lab exposed animals to field collected individuals is a powerful way to validate the in-lab data, and identify the responses that are ecologically meaningful. We conducted three experiments where flounder were exposed to oil contaminated sediment and liver transcriptional profiles were assessed. One experiment was based on clean sediment that was contaminated with oil, the second with sediment collected from the field with preexisting levels of oil contamination, and the third was a collection of flounder from various sites in the Gulf of Mexico with differing levels of historical oil contamination. Using IPA, we compared the transcriptional profiles of the oi-exposed flounder from the different experiments. The results indicate that there is a degree of overlap in the genes and pathways that were identified as affected among the different analyses and demonstrate that laboratory exposures provide useful information about field events.

**451 Impacts of oil exposure in early and later life stages of marine fish**

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Impacts on cardiac function and development in early life stage fish are commonly reported effects of crude oil toxicity. Such impacts include pericardial edema and reduced stroke volume and cardiac output. However, additional sensitive endpoints in early life stages include accelerated metabolic rate, accelerated yolk sac depletion, and alterations of buoyancy in developing embryos. Furthermore, brief exposures during embryonic development lead to reduced swim performance and reduced visual acuity in later stage juveniles. Oil exposure results in altered olfactory responses, reduced prey capture ability, and higher susceptibility to predation, in juvenile fish, likely due to altered central nervous system function. Brief, low-level oil exposures, also impacts adult fish leading to reduced aerobic scope and swim performance. The lower swim performance in adult oil-exposed fish, is due to reduced cardiac output driven by reduced stroke volume. Isolated cardio-myocytes show that sarcomere shortening upon electrical stimulation is reduced by acute oil exposure which likely explains the reduced stroke volume observed in intact, oil exposed fish. Such reductions in cardiomyocyte contractility are likely related to impaired cellular calcium cycling also suggested by RNAseq data. Efforts are ongoing to examine the combined effects on sensory systems and the cardiovascular system on habitat utilization and survival in the wild. This research was made possible by a grant from The Gulf of Mexico Research Initiative. Grant No: SA-1520; Name: Relationship of Effects of Cardiac Outcomes in fish for Validation of Ecological Risk (RECOVER).

**452 Taking the laboratory to the field: Differences in migration patterns, habitat use, and spawning behavior of control and oil-exposed wild mahi-mahi**

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Mahi-mahi (*Coryphaena hippurus*, "mahi" in the following) is a highly migratory ecologically and commercially important pelagic fish species that inhabit tropical and sub-tropical waters around the world. The 2010 *Deepwater Horizon* blowout released approximately five million barrels of crude oil into the northern Gulf of Mexico (GOM) and coincided spatially and temporally with the spawning window for mahi. In the laboratory, environmentally realistic oil exposures reduce cardiac performance and aerobic swimming speed of adult mahi. As a high-performance fish, the ecology of mahi is tied to their vertical dives, migrations, and spawning; all of which is poorly studied and may be affected as a result of crude oil exposure. To better understand behavior in wild mahi, we collaborated with Wildlife ComputersÔ to build pop-up satellite archival tags (PSATs) to measure acceleration, depth, temperature, and light levels (for geolocation modeling). To model acceleration patterns around spawning we tagged wild-caught captive mahi with PSATs and observed them in a

30,000 L tank for three weeks. We followed this captive-based experiment with the deployment of 16 PSATs on wild mahi in the Florida straits (n=14) and the GOM (n=2). Wild mahi tagged in the Florida straits generally migrated north and eastward moving up to 100 km per day, while mahi in the GOM remained in the GOM for the duration of the tagging period. Overall, mahi inhabited water temperatures between 31 and 17 °C, explored depths from the surface to 250 m, and spent more time at depth at night than during the day. Exploration of putative spawning events is ongoing. In the summer of 2018 we are conducting an experiment in the GOM where wild mahi will be exposed to concentrations of crude oil observed during the 2010 spill for 24-hours onboard our research vessel, tagged with PSATs, and released into the wild along with tagged control mahi. The spawning, migration, and habitat utilization information from these PSATs will be a vital component of understanding the ecology of mahi and will also provide important information about the fate and performance of mahi exposed to oil in the wild. This research was made possible by a grant from The Gulf of Mexico Research Initiative. Grant No: SA-1520; Name: Relationship of Effects of Cardiac Outcomes in Fish for Validation of Ecological Risk (RECOVER).

**453 Ecotoxicology of Deep Ocean Spills**

*A.C. Bejarano, Research Planning Inc. / Department of Environmental Health Sciences; S. Chiasson, Loyola University / EEB; M.G. Barron, USEPA / Gulf Ecology Division*

Central issues in understanding the risks of subsea oil spills include the unknown sensitivity of deep ocean species, and the complexity and diversity of habitats that may be affected in subsea, offshore, and inshore environments. Unique aspects of the deep ocean relevant to hydrocarbon species sensitivity include the physical environment (cold temperature, hypersaline, no light, high pressure), and biological communities of unknown sensitivity, including deep sea corals and vent communities. Subsea spills entering the photic zone may affect pelagic species and neuston, and result in surface oiling of sea birds, turtles and marine mammals. Risks to pelagic species will vary seasonally with the presence of sensitive life stages in the water column and sea surface. Potentially affected nearshore environments include coastal wetlands, beaches, and estuaries. Relatively little is known about petroleum toxicity to deep ocean species, as well as the diversity and interactions of species in offshore and inshore environments. Approaches for understanding species sensitivity and extrapolating toxicity to aquatic communities include species sensitivity distributions, existing toxicity databases (i.e., the chemical aquatic fate and effects database), and predictive modeling tools (i.e., interspecies correlation estimation models). This presentation will summarize the limitations, uncertainties and assumptions of toxicity estimation approaches, lessons learned from the Deepwater Horizon spill, and the role of ecotoxicology science in informing future response decisions/field assessments.

## Improving Approaches to Assess Risks to Threatened and Endangered Species from Chemical Exposure

**454 Use Versus Usage: A Critical Consideration for Modeling Pesticide Exposure to Federally Listed Threatened and Endangered Species**

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Section 7 of the Endangered Species Act requires federal agencies to insure that any action they authorize, fund, or carry out is not likely to jeopardize the continued existence of any endangered or threatened species. EPA's authorization of pesticide use through its approval of FIFRA pesticide labels is a federal action subject to Section 7 consultation requirements. Should assessment of potential effects to listed species be based on the action authorized by EPA ("pesticide use" as allowed by the product labeling), or should the assessment be based on projected future use of pesticide products based on recent "pesticide usage?" This presentation will compare and contrast the two approaches for a federally-listed California Central Valley species and discuss the relevant considerations of conducting the assessment in the context of a Section 7 consultation.

**455 A refined endangered species risk assessment for aquatic species exposed to malathion**

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The Environmental Protection Agency (EPA) and Fish and Wildlife Service (FWS) and National Marine Fisheries Service (NMFS) (the Services) are close to completing an organophosphate case study for malathion. The case study currently consists of an EPA generated biological evaluation (BE) and a NMFS biological opinion (BiOp) that was based on the results of the EPA BE. The NMFS BiOp puts forth jeopardy determinations for 12 listed species (38 distinct ecologically significant units or distinct population segments), and 38 critical habitats under their jurisdiction. Both the EPA BE and hence the NMFS BiOp for malathion did not move past a simple, highly-conservative screening-level approaches to characterizing risk. In this presentation we will discuss a refined approach to determining risk for aquatic listed species occupying both static and flowing habitat. To generate refined effects metrics, laboratory studies were evaluated for relevance and study quality and data deemed acceptable or supplemental were used to generate taxon-specific concentration-response curves and species sensitivity distributions. In a companion poster (Winchell et al. 2018) the associated refined exposure modeling effort is described. The refined exposure modeling takes advantage of the best nationally available spatial datasets providing the information necessary to improve the parameterization of regulatory aquatic exposure models such as the PRZM landscape and VVWM receiving water models, and thus refine exposure estimates. This exposure modeling approach resulted in species-specific EEC distributions that could be further filtered according to habitat characteristics (flow rate, water body size) and grouped according to contributing watershed characteristics. Combining refined exposure and effects distributions, informed by species life history data and habitat requirements, allow the risk characterization to quickly identify regions within a species range that are likely to experience elevated risk concerns. A weight-of-evidence approach is also described that provides important context to the risk estimates and helps inform the risk characterization conclusions.

**456 A US, field-scale, herbicide spray drift deposition and biological evaluation study: Methods and implications for risk assessment**

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Spray drift from pesticide applications is considered as a potential route of exposure in environmental risk assessment. Typically, spray drift deposition is modeled using terrestrial plant effects endpoint derived from worst-case, full rate direct spray studies, and combined in the risk assessment framework to represent extreme worst-case risk to non-target organisms. The objective of this work was to merge observed plant effects with spray drift exposure in a single study. A 40-acre field-scale, spray drift study was developed to simultaneously measure spray drift deposition, airborne interception, and potential biological effects of an herbicide under conservative drift conditions and a relatively low-drift nozzle. This study was conducted in four replications, each with a two-swath spray pattern (90 ft per swath) upwind of a deposition zone (perpendicular to wind direction), generally following the generic U.S. Environmental Protection Agency verification protocol, *Testing Pesticide Application Spray Drift Reduction Technologies for Row and Field Crops*. Within each replicate application, an array of three perpendicular sampling lines were used to measure drift deposition out to 400 ft, airborne interception out to 75 ft, and potential direct plant effects at set distances (5, 15, 25, 23, and 45 ft) from the edge of the downwind spray application . At each distance and sampling line, further replication of spray drift deposition, airborne interception, and biological effects were assessed in replicated fashion in a nested, replicated design. The timing of the herbicide application for each of the four replications targeted steady wind speeds between 8 to 12 mph. Wind direction was measured within a 30-degree angle of the downwind field orientation to ensure that spray drift would travel toward the collection area and across the furthest sampling points. Results from this study design refine effects determined in laboratory studies under worst-case exposure scenarios (i.e., direct over the top application) by addressing how terrestrial non-target plants actually experience exposure under natural conditions in the field, which can better inform risk assessment and risk management decisions.

**457 Development of a probabilistic co-occurrence analysis approach for listed insect species**

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Defining species distribution as well as pesticide use areas is an important step in understanding potential co-occurrence of listed species and their critical habitats and pesticide use areas. Development of scientifically rigorous approaches to better understand the likelihood of proximal overlap between pesticide application and listed species habitat can improve such efforts. Here we demonstrate a new method of co-occurrence analysis that combines spatial models of pesticide use areas and species distribution in a probabilistic framework. Specifically, we evaluated three components: 1) advance our previously published probabilistic crop footprint methodology (Budreski et al., 2016), 2) develop a habitat modeling approach for listed insect species, and 3) develop a joint probabilistic co-occurrence analysis approach using advanced species and use area data. Using the principles of the Bayes' Theorem, probabilistic spatial models of pesticide use areas were constructed by integrating information from the Cropland Data Layers, National Land Cover Datasets, NASS Census of Agriculture and Quick Stats and all classification accuracy assessments. In addition, we incorporated available pesticide use data (USGS) as well as recent satellite imagery products to improve field boundary delineation. To develop and validate a probabilistic species distribution model, we tested multiple methods by identifying species-specific landscape variables that could serve as predictors for listed species with different life histories and habitat needs. We also parameterized these models with novel predictor variables including newly available remote sensing data products. We tested best-fit distribution models using the available observation records for the species. Combining two refined modeling outputs, we conducted the joint probabilistic co-occurrence analysis by integrating crop presence probability and likelihood of habitat suitability. This work serves as a framework to enhance the accuracy and efficiency of threatened and endangered species assessments using a data-driven likelihood analysis of species co-occurrence.

**458 Developing Integral Projection Models for Aquatic Ecotoxicology**

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Extrapolating laboratory measured effects of chemicals to ecologically relevant scales is a fundamental challenge in ecotoxicology. Structured population modeling seeks to utilize data and dynamics linked to a given trait (or traits) of individuals in the population; age-, stage-, and size-structured modeling approaches are common examples. We present integral projection models as a novel and promising approach to structured population modeling within an ecotoxicological context. We demonstrate the flexibility of this approach to integrate chemical and non-chemical stressors into complicated life-histories by showing how our approach can be adapted to accommodate batch-spawning, over-winter survival, and the effects of time-variable exposure scenarios in Fathead minnow (*Pimephales promelas).* We also discuss how this approach can be adapted to inform the ecological risk assessment of the endangered Delta smelt (*Hypomesus transpacificus*), by incorporating semelparity and spatially distinct metapopulations for different life-stages.

**459 Development of a neurobehavior adverse outcome pathway in larval fish to facilitate cross-species extrapolations**

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To assess the impacts of chemical exposure on threatened or endangered species usually requires making assumptions about animals where there is limited information on which to base risk assessment. Our lab is developing methods to improve cross-species extrapolations so that we can make decisions on data poor species. We report on progress for an approach that we believe has the potential to improve cross-species extrapolations and to bridge essential data gaps so that we can predict effects from molecular/cellular level to population and community level responses on endangered species from experiments conducted on laboratory animals. We found that the adverse outcome pathway (AOP) framework is a highly useful organizing base from which to collect data in a systematic way to facilitate cross- species extrapolations. We are developing a neurobehavior AOP in the zebrafish and we are extrapolating it to two other species of ecological importance, the fathead minnow and the yellow perch. We collected in depth data on yellow perch and fathead minnow that may allow us to predict from molecular initiating evens to cohort survival and growth which are endpoints that are critical for ecological risk assessments. With these data sets we will be able to assess the feasibility of cross-species extrapolations – we will be able to determine what information is useful for cross-species comparisons and what information is lost when a model species is used instead of the target organism and the amount of error associated with cross-species assumptions.

**460 Using plant functional traits to guide herbicide risk assessments**

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Plant functional traits are morpho-physio-phenological traits, which influence fitness indirectly via their effects on growth, reproduction and survival. This makes functional traits very important because they determine how plants will respond to external factors and potentially how a population will respond. Functional traits have been successfully linked with demographic traits for neotropical trees, shrubs, palms and herbaceous plants. There are well-supported relationships between particular functional traits and demographic indices. The present study sought to address two points relevant for risk assessment of threatened and endangered (listed) plants; First, it expands on the available plant functional traits-demography links by identifying functional traits for listed species using demographic and eco-physiological plant databases (COMPADRE and TRY) and literature. This facilitates characterization of functional traits for listed species and generates demographic data that can facilitate conservation management efforts. The second point relates to the use of plant trait characterization to enhance herbicide risk assessments for listed plant species. Surrogate plant test species, which are used to develop toxicological endpoints in risk assessments, can be incorporated in the functional trait characterization in order to provide guidance on which surrogate species to use when developing listed plant risk assessments.

Trait analyses can also inform explorations into the use of new or more reliable surrogates. The main goals of this project are to identify data gaps for listed plant species, which are usually limited on demographic data, and to attempt to “fill them” once identified. This will provide useful data for the development of listed plant risk assessments at the population level.

**461 Combining an individual-based model and an aquatic food web-ecosystem model to assess ecological risks: A case study with the endangered Topeka shiner**

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The Comprehensive Aquatic System Model (CASM) is a process-based integrated bioenergetics and habitat quality model that simulates population, community, and ecosystem-level effects of chemical stressors based on an aggregated population structure defined by an average-sized individual. An individual-based bioenergetics and population model (IBM) was developed for the endangered Topeka shiner (*Notropis topeka*) to incorporate detailed life-history and age-specific and size-specific attributes of population dynamics not represented in the CASM. The models were executed in tandem with daily IBM population growth dynamics transferred to CASM, which in turn computed the corresponding daily modifications to the food web. The CASM food web results were transferred back to the IBM in the form of adjusted Topeka shiner prey biomass values. This uniquely integrated model combination was implemented to simulate potential ecological risks for Topeka shiner in a generalized Iowa headwater pool, representative of known Midwestern habitat and range for this species. Ecological risks were computed using time-integrated differences between the population biomass values of 365-day baseline and exposure simulations. Risks were estimated for example daily pesticide exposures of varying magnitude, timing, and duration. Potential direct toxic effects to Topeka shiners were modelled within the IBM. The resulting modelled impacts on population biomass were used by the CASM to compute corresponding food web-ecosystem effects. The IBM provided the capability to examine the potential population-level risks based on detailed sensitivities of early life stages and adults to pesticide exposures. The CASM extended the IBM assessment capability by extrapolating potential direct effects on Topeka shiners to associated indirect changes in headwater pool community structure and ecosystem function. The presentation highlights the advantages afforded by the integrated IBM-CASM modeling approach to ecological risk assessment.

## Evolutionary Ecotoxicology Then and Now: Decades of Progress in the Science of Evolution to Pollutants

**462 The contribution of detoxification pathways to pyrethroid resistance in *Hyalella azteca***

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Chronic exposure to pyrethroids can result in sublethal impacts to non-target species in aquatic systems, driving population-level changes. Characterizing the underlying mechanisms of resistance is essential to better understanding the role and potential consequences of contaminant-driven microevolution. The current study demonstrates that multiple mechanisms enhance the overall phenotypic expression of resistance characteristics in *Hyalella azteca*. In VGSC mutated *H. azteca*, both adaptation and acclimation traits may have a role in the attenuation of the adverse effects to pyrethroid exposures. Pyrethroid resistance is primarily attributed to the heritable mutation at various loci of the voltage-gated sodium channel, resulting in reduced target-site sensitivity. However, some additional reduced pyrethroid sensitivity can also be conferred through reversible physiological responses to environmental conditions, such as enhanced enzyme-mediated detoxification. Two pathways significantly contributed to detoxification of permethrin in *H. azteca,* including cytochrome p450 monoxygenases (CYP450) and general esterases (GE). Over time, VGSC mutated *H. azteca* retained most of their pyrethroid resistance, though there was some increased sensitivity from parent to offspring when reared in the absence of stressors. The permethrin 96 h LC50 declined from 1809 ng/L in P0 individuals to 1123 ng/L in the F1 generation, though still remained well above the 20.4 ng/L of wild-type individuals. This reduction in tolerance was likely related to alterations in acclimation mechanisms conferring resistance traits, rather than changes to target-site sensitivity. Enzyme bioassays indicated decreased enzyme activity from P0 to F1, whereas the VGSC mutation was retained. The permethrin LC50 values in resistant *H. azteca* were still two orders-of-magnitude higher than non-resistant populations indicating that the largest proportion of resistance was maintained through the inherited VGSC mutation. Thus, some variation in phenotypic expression of resistance characteristics in *H. azteca* over time is likely associated with uninheritable genetic factors or non-constitutively expressed traits controlling enzyme pathways which overlie a strong heritable component of resistance. A better understanding of the mechanistic and genomic basis of variable acclimation will be necessary for better predicting the ecological and evolutionarily consequences of contaminant-driven change in *H. azteca*.

**463 Contaminant-Mediated Selection Alters Gene Flow and Genetic Diversity in Mussels from the Strait of Istanbul**

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Mediterranean mussels (*Mytilus galloprovencialis*) were collected from various sites along the Strait of Istanbul in Turkey. These sites have been the focus of a long-term environmental and biomonitoring project, and the sediment concentrations of polycyclic aromatic hydrocarbons, metals, pesticides, and polychlorinated biphenyls at these sites have been previously characterized. DNA was extracted from these mussels, and a 562 bp-long segment of the mitochondrial control region was amplified by PCR, sequenced, and subjected to various genetic and phylogenetic analyses. The major findings were that 1) a minimum spanning tree identified 3 clades (Clade 1, Clade 2, Clade 3; Clade 3 contained only a single rare haplotype) separated by the nearest clade at least 15 mutational steps, 2) the amount of genetic diversity and frequency of Clade 1 haplotypes was negatively correlated with degree of sediment contamination, while the frequency of Clade 2 haplotypes was positively correlated with the level of environmental contamination, 3) Tajima’s D test indicated that there was balancing selection at the least contaminated site, and purifying selection at the most contaminated site, 4) $\Phi_{ST}$ values and genetic structuring among sites corresponded to levels of contamination. It was concluded that 1) a Pleistocene vicariance event produced the clades, which may differ in tolerance to toxicants, 2) strong directional currents and local selection can produce fine-scaled genetic structure in this species, 3) the was evidence that toxicant susceptibility was associated with D-loop haplotypes, and this affected levels of genetic diversity and gene flow. This study suggests that evolutionary history (e.g. vicariance) may affect differential responses to toxicants among individuals within populations, and this should be taken into consideration in ecotoxicological studies.

**464 Variation in signatures of cadmium-induced mitochondrial DNA mutations in Daphnia pulex reveal evolutionary effects of pollution**

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Understanding the origin, persistence, and consequences of mitochondrial DNA (mtDNA) mutations are increasingly fascinating as recent evidence implicates heritable mtDNA mutations in population level phenotypes as well as organismal health. However, the effect of chemical stressors on mtDNA mutation rates is largely unknown. We use an experimental evolution approach in the freshwater crustacean, *Daphnia pulex*, to investigate variation in germline mtDNA mutation rates of two different populations: one population from a mining-devastated region, and the other from a pristine environment. The genotype from the contaminated region is from an adapted population of *D. pulex* that is tolerant to cadmium (Cd) after a century of exposure to iron ore smelting runoff. We take advantage of this variation in population histories to investigate the effect of Cd on mutation rate, which is a known mutagen. Twelve genetically identical individuals of the tolerant ("T") population and a Cd-sensitive reference population ("S") of D. pulex were exposed to both control, and chronic, environmentally-relevant cadmium conditions for almost 2,000 generations via a mutation accumulation (MA) experiment, followed by deep-coverage, whole-generation sequencing. There is no effect of population history or Cd on overall mitochondrial genome mutation rates. However, there is a trend towards suppression of C:G > A:T transversion mutations and both A:T > G:C and C:G > A:T transition mutations in the "T" MA experimental lines. Strikingly, there are specific context-dependent mutations that occur at C/G sites in the "S" experimental lines, which are absent in the "T" MA experimental lines. We also observe variation in the frequency and region of insertion and deletion mutations, with "T" indels occurring more randomly throughout the genome, with "S" indels occurring at high frequency in the D-loop, or non-coding region, of the mitochondria. Overall, we see low frequencies of mutations, ranging from 3 – 50%, which suggests that either mutations are recent, or that *D. pulex* retain low levels of heteroplasmy. One line of "S" in Cd shows a mutator phenotype, with a mutation rate >100-fold higher than the other experiments, which to our knowledge has not been observed in a mitochondrial genome. Overall, these results suggest variation in the spectrum of mtDNA mutations across different populations under variable environments.

**465 Evolutionary toxicology in practice: Case studies with Daphnia highlight potential regulatory applications**

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Differences in inter-individual response are described by variation in environments and genomes that combine to give rise to phenotypic variation observed in populations. Evolutionary toxicology provides tools for understanding these features that offer links to phenotypes, which are in-line with proposed changes in regulatory toxicology. This talk will draw from laboratory and field investigations, which include long-term mutation accumulation experiments and environmental genomic case studies, to explore how environmental stress contributes to genome variability, influences the fate of genetic variation in populations, and over micro-evolutionary time scales determines the fate of phenotypes. These studies contribute to and make use of maturing genomic tool kits for the water flea, *Daphnia pulicaria*, including new reference genomes. Using this animal model, we explore how stress shifts the genome distribution of mutations and changes the rate of specific mutational classes –including those that alter epigenetic state. We discuss how genome variation drives functional variation in gene expression and gene regulatory networks, and contributes to organismal fitness. We explore how environment-induced alterations in the magnitude and distribution of mutations, including gene copy number variation (CNV), in natural populations contributes to adaptations to extreme environments. These findings, which demonstrate the importance of understanding genome variation and the evolutionary forces that shape it, provide answers that can help fill regulatory needs. The field provides tools for identifying molecular pathways that contribute to adverse outcomes and predicting the fate of populations. After decades of research these tools are catching pace with the complexity of the problems, however, their output has not been directed towards meeting regulatory needs. Perhaps, it is time to move the discussion from evolutionary toxicology as an academic pursuit to its role in protecting human health and the environment.

**466 Evaluating wastewater effluent exposure in wild fishes using insights from laboratory and wild populations**

*D. Winkelman, J.R. Anderson, Colorado State University / Fish, Wildlife and Conservation Biology; A.M. Vajda, University of Colorado Denver / Integrative Biology; A. Jastrow, USEPA / Region 5*

Complex chemical mixtures in effluents below municipal wastewater treatment plants (WWTPs) are difficult to assess and their effects on organisms are poorly understood. In the South Platte River basin in Colorado, estrogenic impacts from treated municipal wastewater have been observed, but there is limited data about the consequences of estrogen exposure for wild organisms. Our goal was to evaluate several species of wild fish for evidence of estrogen exposure and to determine if the effects observed in laboratory raised Fathead Minnow (FHM, *Pimephales promelas*) caged in wastewater were similar to those observed in wild fish populations. To accomplish this goal, we placed ten laboratory raised male FHM in cages both upstream and downstream of a WWTP effluent for one week, and then euthanized them, and extracted their livers for Vitellogenin (VTG) analyses. The presence of VTG is a common biomarker for estrogen exposure in male fish, and VTG presence was determined using qPCR. We also captured wild fish species (*Catostomus commersonii*, *Rhinichthys cataractae*, and FHM) at the WWTPs where we caged FHM, and measured VTG protein in wild fish blood plasma using ELISA. Finally, we caged wild FHM at the same locations in which we caged laboratory raised FHM and measured VTG production. Analysis for wild fish VTG data is ongoing, but our initial results indicate that wild FHM captured at the caging sites did not express similar levels of VTG as their caged laboratory raised counterparts. Additionally, caged wild FHM expressed more VTG than unconfined wild FHM, but less VTG than the laboratory raised FHM caged at the same spot downstream of the WWTP effluent. Our results indicate that the response of VTG production in laboratory raised FHM is more sensitive to estrogen exposure than VTG production in wild FHM, demonstrating that wild FHM seem to be acclimated or adapted to living in WWTP effluent compared to the naïve laboratory population. These results have consequences for the potential inferences being made between laboratory and wild populations and indicates that more research needs to be done to understand the differences between wild and laboratory raised populations.

**467 Genetic Architecture of Pollution Resistance in Multiple Populations of Killifish**

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Multiple populations of Atlantic Killifish (*Fundulus heteroclitus)* that reside in heavily polluted habitats along the Atlantic coast of North

America have repeatedly and rapidly evolved resistance to highly toxic dioxin-like pollutants (DLCs), which act through the aryl hydrocarbon receptor (AHR) signaling pathway. A multi-population genome-wide scan showed that wild DLC-resistant killifish populations have some shared and some unique genomic regions under selection. These studies and others suggest that resistance to the toxic effects of DLCs, such as cardiovascular and other developmental deformities, is extreme but not identical in resistant killifish populations. Here, we extended the QTL (Quantitative-Trait Locus) approach to four resistant killifish populations distributed from Massachusetts to Virginia, and employed high-density QTL interval mapping to compare and contrast genetic regions associated with DLC resistance in these diverse populations. Our results show that in all four populations, a common genomic region associates with DLC resistance. This region includes two AHR pathway genes (AHR1b/2b) that did not show obvious signatures of selection in population genome scan data. Conversely, another AHR pathway gene (AIP) falls in a genomic region showing strong signatures of selection and associates with resistance in three of the four of our mapping populations. These results also suggest that variation in AHR1b/2b may have evolved by soft sweeps on standing genetic variation in resistant populations, which are difficult to detect by traditional selection scan methods. These results also suggest that AIP is an important component in the evolutionary response to complex, heavily contaminated environments. Our findings, integrated with population genomic scans for selection, reveal the unique evolutionary trajectories that led to evolved DLC resistance in wild killifish.

**468 Metabolic tradeoffs of pollution adaptation in teleost Fish Fundulus heteroclitus**

*N. Jayasundara, Universiy of Maine / School of Marine Sciences; R.T. Di Giulio, Duke University / Nicholas School of the Environment*

Anthropogenic stressors, including chemical pollutants, are key evolutionary drivers in the environment. However, adaptive changes in fundamental physiological processes in response to anthropogenic selection pressures may have significant consequences (e.g., energy metabolic tradeoffs), but remain poorly characterized. Atlantic killifish in the Elizabeth River, Virginia have evolved resistance to a ubiquitous class of pollutants, polycyclic aromatic hydrocarbons (PAHs), and provide a unique “natural-experiment” to examine metabolic tradeoffs of rapid evolution to anthropogenic pollution. Here we focused on our ongoing and published studies and examined data from population genomics analyses, genome-wide methylation analyses, gut microbiome studies, metabolomics studies and whole organismal and developmental bioenergetics assays to determine shifts in energy metabolic processes in PAH-resistant fish. Metabolic phenotype of PAH-resistant fish is compared to fish from a clean site (Kings Creek- KC). Genomic data indicate that genes associated with cellular energy metabolism, especially mitochondrial function, are likely under selection in fish from PAH-contaminated sites. Physiological studies reveal that mitochondrial function is altered in adult tissues and in developing embryos in PAH-resistant fish. In particular, organismal and embryonic metabolic rates are higher in PAH-resistant fish compared to KC fish. However, despite elevated mitochondrial oxygen consumption rates, metabolomics data show overall suppression of metabolic products including amino acids and sugars, suggesting reduced metabolic turnover. In addition, shifts in sphingolipid metabolite levels as well as the reduced levels of sphingolipid producing bacteria in the PAH-resistant fish gut. These observations parallel the population genomic data showing that genes associated with fatty acid metabolism, including sphingolipid metabolism are under selection. Collectively, our studies demonstrate that pollution adaption and inhabiting contaminated environments may compromise energy metabolic processes in *Fundulus heteroclitus,* and these changes in energy metabolism are likely to be associated with shifts in the gut microbial community.

**469 Adaptive introgression enables evolutionary rescue from extreme environmental pollution in Gulf killifish**

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Anthropogenic contamination associated with industrial activity is a widespread and active threat to the stability of populations. The Houston Ship Channel (HSC) is one example of a heavily impacted environment, where industrial activity has contributed to extreme levels of pollution, including polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs), polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). This study explores the impacts of chronic multi-generational exposure to industrial pollution on the physiology, demography and population genomics in a keystone coastal species – Gulf killifish (*Fundulus grandis*). We have characterized population-wide sensitivity to contaminants in fish from 12 locations across Galveston Bay, as well as the contamination levels at those sites. We found a gradient of chemical resistance that was positively correlated with contaminant concentrations. This resistance was also correlated with a suppression of the aryl hydrocarbon receptor (AHR) signaling pathway, as estimated via the activity of a down-stream regulated enzyme – cytochrome P450 1A (CYP1A). We showed that the heritability of this resistance is biparental and multi-generational. Finally, we performed full genome resequencing of many individuals from each of seven populations along this gradient of resistance and discovered that genomic regions under selection in adapted populations included several genes that regulate the AHR pathway. In addition, some of the adaptive variants in resistant *F. grandis* have been acquired through introgression of DNA from southern populations of *F. heteroclitus* within the last 50 years. To our knowledge, this represents the first case of evolutionary rescue from anthropogenic contamination through adaptive introgression and suggests that introduction of non-native species may sometimes deliver genetic variation that enables adaptation of native species to rapidly changing environments.

## Alternative Animal Ecotoxicity Testing: New and Novel Approaches for Predicting Environmental Hazards and Risk Assessment

**470 Developing Weight of Evidence Arguments for Assessing the Acute Toxicity of Chemicals to Fish**

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Acute responses of fish to chemicals is generally assessed by an OECD Test Guideline 203 method or equivalent. Animal welfare concerns emerged in the last 2 decades that have resulted in the development of several new methods including fish gill cytotoxicity and fish embryo toxicity tests. In 2013, OECD TG 236 Fish Embryo Toxicity (FET) assay for the estimation of acute fish toxicity was validated and adopted at OECD. A recent regulatory opinion rendered by the European Chemicals Agency challenged ecotoxicologists to develop a holistic weight of evidence (WoE) approach that includes FET and other considerations to address requirements for understanding acute fish toxicity to registered chemicals. In this talk, we review recent developments including the status of knowledge on FET and prediction towards acute fish toxicity, FET and fish QSARs, Fish Threshold Approach thinking, the role of mode of action

(MoA) assignment, and progress in a comprehensive Bayesian Belief Network devoted to WoE that incorporates FET data. FET data continues to be developed for all types of compounds. This has occurred even after the assertion that FET under-predicts neurotoxic compounds relative to fish. Integration of all lines of evidence, ranging from the several hundred FET and corresponding fish data from a wide range of chemicals and MoAs, can point to high likelihoods of predictivity of the FET for many classes of compounds. QSARs based on FET results are particularly robust for neutral organic, phenolic, analine, amine, and charged surfactant compounds (non-polar and polar MoA) and are quantitatively indistinguishable from acute fish QSARs. Chemical classes and MOA that are under-represented in the FET and fish toxicity databases regarding high quality OECD TG 203 and TG 236 studies provide particular challenges including neurotoxic and other bioactive or specifically-acting compounds. Vexing issues, such as the quality of OECD 203 data used as the reference standard to judge validity of the OECD 236, studies lacking analytical (in one or both of the OECD 203 or 236), test chemical solubility, and mode of action assignment remain as problems which will take concerted efforts to solve. That said, these can be addressed by WoE arguments which will be summarized as a proposed process diagram in this talk.

**471 Advancing predictive QSAR and AOP development: 3-dimensional protein modeling, molecular docking, and structure-based pharmacophore approaches**

*M.G. Barron, USEPA / Gulf Ecology Division; S. Lee, Daegu-Gyeongbuk Medical Innovation Foundation*

Traditional approaches to quantitative structure activity relationship (QSAR) modelling have used either single physical-chemical descriptors such as log-Kow or multiple two-dimensional theoretical molecular descriptors. The increasing availability of protein crystal structures and advances in computational chemistry now allow efficient conformer generation and docking in three-dimensional (3D) protein models. 3D QSAR models were developed for aromatase, acetylcholinesterase, and the estrogen receptor. For each model, 3D-fingerprint descriptors encoding protein-ligand interactions were developed using molecular docking and structure-based pharmacophores to rationalize the structural requirements responsible for the activity of a diversity of agonist and antagonist ligands. In silico mutagenesis was also used to understand differences in species sensitivity due to amino acid substitutions at key molecular interaction points. The results show that 3D QSAR models can serve as a predictive framework for quantitative and mechanistic understanding of complex molecular initiating events, and define the chemical applicability domain of specific Adverse Outcome Pathways.

**472 Leveraging Zebrafish to Identify Chemicals Disrupting Early Embryonic Development**

*S. Vliet, S. Dasgupta, D.C. Volz, University of California, Riverside / Environmental Sciences*

Alternatives to conventional toxicity testing are needed to support chemical screening and prioritization. Zebrafish embryos offer one of the most promising cost-effective vertebrate models to support toxicity testing. Therefore, the overall objective of this project was to leverage early, non-protected life stages of zebrafish embryos to identify compounds that disrupt the normal trajectory of early embryonic development. For the first aim, we carried out a high-content screen of the LOPAC[1280] (Library of Pharmacologically Active Compounds) library – a commercially available library of 1,280 marketed drugs, failed development candidates, and well-characterized small molecules widely used for validation of high-throughput screening assays. Based on this screen, niclosamide – an antihelminthic drug used worldwide for the treatment of tapeworm infections – was one of the most potent developmental toxicants within zebrafish embryos during the first 25 h of development, with exposure to 10 µM niclosamide from 5-25 h post-fertilization (hpf) resulting in 100% embryo mortality. Therefore, the second aim of this study was to investigate the mechanism of toxicity of niclosamide during early stages of embryonic development. We found that niclosamide induced a concentration-dependent delay in epiboly progression during late-blastula and early-gastrula, an effect that was dependent on exposure during the maternal-to-zygotic transition – a period characterized by zygotic genome activation and initiation of cell motility. Moreover, we found that niclosamide did not affect embryonic oxygen consumption, suggesting that oxidative phosphorylation – a well-established target for niclosamide within intestinal parasites – may not play a role in niclosamide-induced epiboly delay. However, mRNA-sequencing revealed that niclosamide exposure during late-blastula and early-gastrula significantly impacted the abundance of cytoskeleton- and cell cycle regulation-specific transcripts. Indeed, we found that niclosamide inhibited tubulin polymerization in vitro, suggesting that niclosamide-induced delays in epiboly progression may be driven by disruption of microtubule formation and cell motility within the developing embryo. Overall, our findings highlight the utility of embryonic zebrafish as a physiologically-intact, non-mammalian model for screening and prioritization of chemicals and environmental samples for further testing within rodents and human cell-based systems.

**473 Characterization of the gill cell line RTgill-W1 as an in vitro alternative to in vivo acute WET testing methods**

*J. Scott, ATC Group Services / Integrative Biology; M. Minghetti, J.B. Belden, Oklahoma State University / Integrative Biology*

Current acute Whole Effluent Toxicity (WET) test methods are quite laborious, not very cost effective, and lack detail in identifying a toxicant's mode of action. Fish cell lines offer an inexpensive, high throughput approach, and simultaneous measurement of cellular toxicological endpoints such as metabolic activity, membrane and lysosomal integrity. Due to major roles the gill serves in fish physiology, the cell line RTgill-W1, was evaluated as an in vitro alternative model for acute vertebrate WET testing. Exposure conditions require considerations as RTgill-W1 cells cannot be exposed directly to water samples and require modification of osmolarity through addition of salts to the exposure medium which can affect bioavailability of toxicants. The first step of this study was to compare the 50% effective concentration (EC50) of 14 selected toxicants (commonly found in effluent samples) in isosmotic conditions and correlate them to the 50% lethality concentration (LC50) in freshwater (fathead minnow, *Pimephales promelas*) and marine (sheepshead minnow, *Cyprinodon variegatus*) species from literature. Measured chemical concentrations were confirmed analytically for all exposure conditions. Secondly, we determined cell tolerance to medium of varying osmolality (50 to 1000 mOsm/Kg). In isosmotic exposures 13 of the 14 toxicants tested, showed a significant correlation between in vitro cytotoxicity EC50s and in vivo literature LC50s of both fish species. Cells tolerated osmolality from 150 to 450 mOsm/Kg. Thereafter, cytotoxicity of a subset of chemicals was tested in hyposmotic, and hyperosmotic conditions. Cytotoxicity of Cu was increased by hyperosmotic conditions, and by both hypo- and hyper- osmotic conditions for Ni. Although, these adjustments mimic environmental conditions more closely, they do not represent the actual osmolality of effluent water. Therefore, we are currently investigating whether RTgill-W1 cells cultured on permeable membranes or transwells can tolerate a broader range of media osmolality (5 -1000 mOsm/Kg). Concluding, the ability to correlate in vitro EC50s to in vivo LC50s of several chemicals is a crucial first step in establishing RTgill-W1as an alternative acute WET testing model. Moreover, by determining if RTgill-W1 cells can tolerate direct exposures of various osmotic condition will strengthen this in vitro model's applicability in routine acute WET testing.

**474 Metabolite Profiling of Zebrafish Liver Cell Culture for Environmental Monitoring and Surveillance**

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Anthropogenic chemicals released from waste water treatment plants, as well as industrial and agricultural operations often negatively affect surface water quality. It has been shown in previous studies that exposure to such complex chemical mixtures can cause adverse health effects in ecological organisms. Traditional methods using live animals (e.g., fish) for monitoring and assessing contaminant exposure and impacts in affected ecosystems are both resource and time intensive and thus often impractical for screening large numbers of impacted sites. Cell-based metabolomics is proving useful for conducting rapid assessments of environmental exposures, providing a means to reduce animal use and the associated resource investment. In addition, cell-based metabolomics tools are typically unbiased (or untargeted) thus providing an evaluation of biological effects that may not be monitored for when using other techniques. Finally, a large variety of cell types (both human and otherwise) may be employed to investigate impacts across species and/or in specific tissue types. However, the ability to extrapolate metabolite changes determined in vitro to those that would be anticipated to occur in vivo is an ongoing challenge. In this study, we applied recently developed in-house high-resolution liquid-chromatography mass spectrometry (HR LC-MS) methodologies for detecting hydrophilic and lipophilic metabolites in both zebrafish liver cell cultures (ZFL) and zebrafish liver tissues to assess similarities and differences in the detectable metabolomes. Here we report the results of these analyses, with specific emphasis on those biochemical pathways and networks that are shared (and of greatest potential for making in vitro to in vivo extrapolations) and those are specific to each system. Such an evaluation is a critical step in the development of cell-based metabolomics for environmental monitoring of impacted surface waters.

**475 Use of cell-based models to investigate xenobiotic metabolism of pollution-adapted Gulf killifish (*Fundulus grandis*) from the Houston Ship Channel, TX**

*M.E. Franco, C.W. Matson, R. Lavado, Baylor University / Environmental Science*

High concentrations of dioxin-like compounds have been measured in the Houston Ship Channel (HSC), Texas, USA. However, the Gulf killifish (*Fundulus grandis*), a ubiquitous species inhabiting the HSC, has been recently characterized to be pollution-adapted. Previous studies in the sister species *Fundulus heteroclitus* have suggested that such adaptation is the result of reduced metabolism, mediated by the reduced activity of the aryl hydrocarbon receptor (AhR) pathway and the subsequent downregulation of CYP450 enzymes. To further characterize pollution-adapted populations and to obtain insight into the biochemical mechanisms driving this adaptation, a comparison of ethoxyresorufin-O-deethylase (EROD) activity, as a proxy for CYP1A expression, was conducted between liver microsomes and two established fish liver cell lines. This was based under the hypothesis that low microsomal but high cell-based EROD activity would be observed for pollution-adapted populations. Adult fish were collected from heavily polluted and reference sites, and in situ removal of the liver was done immediately after collection. In the laboratory, microsomal EROD activity was measured and compared between adapted and reference populations. In spite of large differences in exposure to AhR agonists, microsomal EROD activity was not significantly different among these populations, suggesting that the expression of metabolizing enzymes might be reduced in adapted populations. Furthermore, pressurized liquid extractions were obtained from fish tissue from both adapted and reference populations, with the aim of obtaining accumulated AhR agonists, and the fish liver cell lines PLHC-1 and Hepa-E1 were exposed to these extracts for 48 h. EROD activity in Hepa-E1 cells was significantly higher after exposure to fish extracts from polluted sites than to extracts from reference fish, but no differences in EROD activity were observed for PLHC-1 cells. The present study highlights to major observations: 1. pathways regulating xenobiotic metabolism in pollution-adapted populations may indeed be reduced as a mechanism of adaptation to high levels of industrial pollution, and 2. the selection of appropriate cell-based models is imperative when used as tools in toxicological studies and for specific research purposes. The use of in vitro models proves to be advantageous in describing adapted populations of fish and, potentially, other species inhabiting highly polluted environments.

**476 Strategically Selecting Test Species using the Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) Tool**

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Chemicals in the environment can affect the health of wildlife with the potential for vastly different sensitivities among species. However, toxicity data used in risk assessment is based on a small number of model test species which might not represent the diversity of species sensitivities. This uncertainty is particularly true for many contaminants of emerging concern (CECs), including agonists of the peroxisome proliferator-activated receptor y (PPARy), for which species-sensitivity information might only be available for one or a few species. However, performing toxicity testing for many species to determine the range in sensitivity is not practical. Therefore, the goal of this study was to demonstrate a means of selecting test species in a strategic fashion to demonstrate a computational means of selecting species for toxicity testing for characterizing differences in sensitivity. Specifically, this study used the Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) tool to rapidly and computationally predict species-specific chemical susceptibility across phylogenetically diverse species through evaluation of structural similarities and differences in the protein target of a chemical. The public literature was used to identify 17 key amino acid residues in the ligand binding domain of PPARy that interact with chemicals, along with knowledge of amino acid differences that cause differences in binding affinity. Differences in these amino acids were investigated among sequences of 246 phylogenetically diverse species using SeqAPASS. Four amino acid positions had differences across species that were predicted by SeqAPASS to potentially result in differences in binding of chemicals and therefore might be important in differences in sensitivity among species. Five PPARy-types were proposed that likely differ in their sensitivity to agonists, namely Type 1 (mammals), Type 2 (birds, reptiles, amphibians, ancient fish), Type 3 (most fish), Type 4 (salmonids), and Type 5 (zebrafish). Based on these results, Xenopus (Type 2), fathead minnow (Type 3), rainbow trout (Type 4), and zebrafish (Type 5) were strategically selected as being representative of the diversity of species sensitivities to agonists of PPARy for ongoing investigation. This study demonstrates how SeqAPASS can be used to computationally predict species most likely to differ in susceptibility to chemicals for the strategic characterization of species differences in sensitivity.

**477 Identification of innate immunotoxicity of perfluorooctanoic acid (PFOA) in zebrafish embryos using in situ hybridization**

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Perfluorooctanoic acid (PFOA) is ubiquitous and persistent in the environment and can be found in nearly all environments across the globe. PFOA is detectable in most human serum and environmental media, and is expected to persist given the relatively long half-life. PFOA has been classified as persistent and bioaccumulative, and was recently labeled "*presumed to be an immune hazard to humans*" by the NTP. However, the exact immunotoxicity mechanisms for PFOA have yet to be fully elucidated, and little data exist on the potential for PFOA to modulate innate immune responses or to alter ecological immune responses. Therefore, we investigated the overt innate immune effects of PFOA exposure in zebrafish embryos (ZFEs). ZFEs were exposed to PFOA for 48 hours and the neutrophil chemotaxis assay was applied to stimulate neutrophil chemotaxis and migration to the site of inflammation. Neutrophils were stained using in situ hybridization for the myeloid peroxidase (MPX) protein, and total number of neutrophils were counted in the wounded region across treatments. We were able to show a significant reduction in neutrophil chemotaxis to the wound site in PFOA treated embryos as compared to control for both the low and high dose exposure. Potential mechanisms for this effect could include an overall systemic neutropenia from altered granulopoiesis or alterations to chemotaxis/chemokine signaling, and are currently being explored. Because there is very little information investigating immune effects during early development, windows of susceptibility, or on the ecological impacts of immune modulation, these data are useful in identifying an adverse immune phenotype in a standard ecological model with high relevance to human health. Use of a single model that can be used to identify both ecological and human health effects contributes to the 3Rs aimed at reducing animal testing. Future studies (in progress) will evaluate the mechanistic and genetic pathway modulation in the adverse outcome pathway related to the identified innate immune effects.

## Selenium Ecotoxicology and Management Going on 40: What Do We Know and How Can We Apply It?

**478 Sensitivity of Sturgeon to Copper, Cadmium, Selenium and Zinc**

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The sensitivity of freshwater sturgeon to copper (Cu), cadmium (Cd), selenium (Se) and zinc (Zn) relative to other fish species has drawn the attention of conservation and environmental groups dedicated to restoring sturgeon populations (Acipenseridae) in the U.S. Sturgeon populations have dramatically declined in the 20$^{th}$ century. There is a general consensus that this decline is primarily due to the loss of spawning habitat (siltation) and placement of dams on rivers preventing the return of sturgeon to spawning grounds. However, there is concern that selenium may be limiting reproductive success for green and white sturgeon in San Francisco Bay and that Cu, Cd, and Zn may play a similar role for white sturgeon in the Columbia River and in rivers on the Great Lakes (lake sturgeon). This presentation reviews the available acute and chronic toxicity (aqueous exposure) of Cu, Cd, and Zn to sturgeon species in comparison to other sensitive fish (and invertebrate) species and US Environmental Protection Agency (USEPA) water quality criteria (WQC). The toxicity of dietary selenium to sturgeon and proposed thresholds (egg/ovary, whole body, muscle) relative to those selected by USEPA for species other than sturgeon is reviewed. Briefly, the data show that juvenile sturgeon (30-60 days post-hatch) are one of the more sensitive fish to Cu, Cd, and Zn, but not always the most sensitive. WQC adjusted for bioavailability appear to be protective for sturgeon for these metals. Regarding selenium, green sturgeon have been shown to be more sensitive to dietary selenium fed as selenomethionine than white sturgeon. Fish collected from San Francisco Bay have been reported to contain ovary concentrations that exceed the EPA WQC ovary threshold of 15.1 ug/g dry wt. for sensitive fish. The extent of selenium toxicity data available for assessment will be reviewed.

**479 Reproductive effects of selenium in the fathead minnow (*Pimephales promelas*)**

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Certain aquatic systems are prone to increased loading of the essential trace element selenium (Se) because of natural sources or anthropogenic activities. All animal classes are susceptible to the toxic effects of Se due to the narrow range between essentiality and toxicity; however, egg-laying vertebrates are at especial risk because of maternal transfer of Se. The objective of this study was to characterize the maternal transfer of dietary selenomethionine (SeMet), the primary form of Se in contaminated aquatic food webs, and its effects on the F1 generation in a short-lived fish species native to North American freshwater systems, the fathead minnow (*Pimephales promelas*). Twenty breeding groups (3 females:2 males) were exposed to dietary SeMet at Se-normal levels (1.18 mg Se/kg food, dry mass [dm]) and environmentally relevant levels (3.88, 8.75 or 29.58 mg Se/kg food dm) and bred for 28 days. Embryos were collected daily and total Se concentrations were measured via ICP-MS to establish the kinetics of maternally transferred Se. Embryos collected on days 26, 27 and 28 were reared to swim-up and assessed for hatchability, survival and morphological abnormalities (frequency, type and severity). Upon onset of exposure an immediate deposition of Se into embryos was observed. After 28-days of exposure, embryo Se concentrations were elevated in a dose-dependent manner resulting in a significant difference in mean embryo Se concentrations from the control (1.80 mg Se/kg embryo dm) in the medium (11.74 mg Se/kg embryo dm) and high (27.17 mg Se/kg embryo dm) treatment groups. Exposure to dietary SeMet did not affect fecundity rates among treatment groups. There was no effect of Se exposure on hatchability or survival among treatment groups. Preliminary deformity assessment revealed an increasing, although not significant, trend in the frequency of deformities between the control and high treatment groups ($p=0.062$). To investigate an alternative exposure route as a proxy for maternal transfer, embryo microinjection of fathead minnow embryos was utilized to deliver Se dosages of concentrations analogous to the dietary maternal transfer study. A suite of biological endpoints from the microinjection study and dietary maternal transfer study will be compared to determine if embryo injection is a valid proxy for maternal transfer. If valid, the injection exposure route could be used to support Se research in early life stages of non-model species.

**480 Moving Forward: Implementation of Tissue-Based Selenium Criteria in Absence of Guidance**

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EPA's tissue-based selenium criteria were finalized in 2016. Soon thereafter, EPA produced draft implementation guidance documents to assist states and dischargers with interpreting the tissue-based criteria and application of the criteria into states' water quality standards and NPDES permits. However, to-date, these documents have still not been finalized, leaving states with no clear guidance from EPA on how to proceed with adoption and implementation of the tissue-based criteria. This lack of guidance has led to states being hesitant to move forward with the adoption of criteria, given existing uncertainty. GEI has been providing technical support for clients in multiple states for the development of statewide or site-specific tissue-based selenium standards, work is currently ongoing in California, Colorado, Minnesota, Mississippi, Iowa, and Indiana. Many questions have been raised regarding implementation of tissue-based criteria during discussions with the various state agencies and regional EPA offices. In this presentation we will review some of the issues and challenges that have arisen in these discussions, such as fishless streams, underlying geology, resident species, and a disconnect between water and tissue concentrations. We will also discuss some lessons learned, and will present practical considerations for parties interested in developing site-specific criteria.

**481 Laboratory-derived selenium toxicity thresholds for fish and evidence of population-level impacts**

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There have been some significant recent developments in regulatory guidelines for selenium in North America, specifically: the finalization of the USEPA criteria for selenium (water and fish tissue), the revision of British Columbia aquatic life guidelines (various media), and selenium limits in the proposed federal coal mining effluent regulation in Canada. Based on field collection of fish gametes, and rearing of fertilized eggs in the laboratory, selenium-related reproductive effects have been documented in both the US and Canada. The toxicity data from field-based exposures, together with toxicity data from laboratory-based exposures, were used to develop the criteria and guideline values noted above. While there have been historical accounts of fish population extirpations in the United States over 30 years ago (e.g., Hyco Lake, Belews Lake), those examples were characterized by high-selenium inputs and selenium concentrations in fish tissue that were much higher than current criteria and guideline values. Similar evidence of population-level impacts on fish populations due to elevated selenium concentrations in the receiving environment have not been demonstrated recently in the US or in Canada. We conducted a meta-analysis of fish selenium concentrations in the field that exceed current fish tissue-based selenium criteria and guidelines, and evaluated whether there were corresponding evaluations of population-level effects due to selenium. Based on this review, fish population assessments are not routinely conducted in receiving waters where fish tissue-based selenium criteria or guidelines are exceeded, but, where they have been, the available data indicate that conclusive population-level impacts have not been observed. This indicates that recent fish tissue-based selenium criteria and guidelines are conservative and protective of fish populations. It should be noted that the more recent cases of tissue-based selenium criteria and guideline exceedances, however, are much more moderate than was observed at Hyco and Belews lakes, for example, which may make potential population-level effects more difficult to detect. The available data and associated uncertainties will be discussed.

**482 Nonlethal selenium monitoring in San Francisco Bay white sturgeon: Factors influencing tissue concentrations and implications for long-term monitoring**

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Selenium is an essential trace element that is toxic at high levels and has been shown to adversely impact reproduction in white sturgeon. To protect this sensitive species, in 2016 the San Francisco Bay Regional Water Quality Control Board established the North San Francisco Bay Selenium TMDL. The TMDL established a target of 11.3 ug/g dw in white sturgeon muscle tissue. In 2015-2017, the Regional Monitoring Program for Water Quality in San Francisco Bay (RMP) conducted two studies to develop non-lethal monitoring methods to assist with TMDL implementation. The first study evaluated relationships between selenium in non-lethally collected muscle plugs and other tissues, including muscle fillet (more commonly used for regulatory monitoring) and ovary and liver tissues (more toxicologically relevant). Tissues were collected from angler-caught adult white sturgeon during a fishing derby held in January-February in North Bay. Results established that muscle plugs are good proxies for muscle fillets in selenium bioaccumulation monitoring, and suggest that plug selenium can also be used as a general indicator of ovary or liver selenium concentration. The second study piloted methods for muscle plug sampling in live sturgeon and selenium analysis in small plug samples. Muscle plugs were collected from 30-60 live white sturgeon annually during fall California Department of Fish and Wildlife sturgeon surveys in the North Bay. Muscle plug selenium concentrations were significantly lower in fall 2017 compared to 2015-2016, immediately following an extended drought period from late 2011 to spring 2017. This finding is consistent with observed patterns in *Potamocorbula amurensis*, a major sturgeon prey item, which have lower selenium concentrations during periods of high freshwater inflow. Significantly lower selenium concentrations were also observed in juvenile compared to adult sturgeon, although no significant pattern was observed among adults across fish lengths. Selenium data were also analyzed in the context of data from previous studies of Bay white sturgeon, to evaluate the effect of additional factors potentially influencing sturgeon selenium concentrations. Freshwater inflow, reproductive maturity, and foraging location were identified as factors driving the high level of variability observed in sturgeon selenium concentrations. This information was used to design a long-term muscle plug monitoring program to assess trends and attainment of the TMDL target.

**483 Characterization of selenium trophic transfer in a boreal lake food web using a whole ecosystem approach**

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Human activities such as coal and metal mining have increased the release of selenium (Se) to aquatic environments, but information about the trophic transfer dynamics of Se in Canadian boreal lake systems is lacking. The goal of the present study was to characterize the uptake and trophic transfer of Se after addition to in situ enclosures located in Lake 114 at the International Institute for Sustainable Development – Experimental Lakes Area (IISD-ELA) in northwestern Ontario. In June 2017, Se was added to 3000 L littoral enclosures as sodium selenite to reach nominal concentrations of 1 µg/L (actual concentration (mean ± SD) = 1.01 ± 0.15 µg/L) and 10 µg/L (actual concentration = 9.58 ± 2.63 µg/L) in triplicates for 77 d, and three additional enclosures were controls with no Se added (background concentration = 0.28 ± 0.04 µg/L after 77 d). Ecosystem level characterization of exposure and trophic transfer were determined by measuring total Se (TSe) concentrations in water, sediment, periphyton, benthic macroinvertebrates, and female fathead minnows (*Pimephales promelas*; added on d 33) collected at the end of the exposure period. Mean (± SD) periphyton Se concentrations after 77 d were 2.95 ± 0.71, 11.92 ± 2.52, and 71.36 ± 18.06 µg/g dw in control, 1 µg/L and 10 µg/L treatments, resulting in enrichment functions (EFs) of 11891 ± 1754, 12142 ± 3208, and 7431 ± 2292, respectively. Se in surface sediments was higher in the 10 ug/L treatment (21.90 ± 4.74 µg/g dw) than in the control and 1 µg/L treatment (2.58 ± 0.58 and 3.68 ± 1.74 µg/g dw, respectively). Trophic transfer factors (TTFs) for benthic invertebrate taxa ranged from 0.6 for Gammaridae to 3.5 for Chironomidae over all treatments. Fathead minnow ovary Se concentrations were 6.91 ± 0.45, 10.87 ± 1.04, and 45.51 ± 2.20 µg/g dw in control, 1 µg/L and 10 µg/L treatments, respectively. Se was enriched by up to four orders of magnitude by periphyton, and Se in fish ovaries accumulated to concentrations near or above the current USEPA criterion (15.1 µg/g dw for fish ovary/egg) at 1 and 10 µg/L exposure levels, suggesting that Se has the potential to accumulate to levels of concern at these concentrations and could pose a toxicity risk to sensitive organisms (i.e. fish and birds) in colder water ecosystems like Canadian boreal lakes. In addition, differences in Se concentrations among potential prey items of fish may influence Se accumulation in lake food webs and warrant further study.

**484 Trophic Considerations: Assessing the Trophic Transfer of Selenium to an Amphipod (*H. azteca*) Through A Diet of Field-Collected Biofilm Communities**

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Understanding the mobilization and accumulation of selenium in the environment is a growing global priority. Toxicological concerns exist particularly due to the detection of devastated oviparous vertebrate populations, triggered primarily by the teratogenic effects of selenium exposure. Natural and anthropogenic sources, and site-specific differences in biogeochemistry result in different concentrations, species, and bioavailabilities of selenium in aquatic environments. Inorganic forms of selenium (eg. selenate [SeO4?²] and selenite [SeO3?²]) are biotransformed and bioaccumulated by microorganisms into organoselenium compounds, which are transferred to higher trophic levels principally via dietary pathways. The present study quantified the trophic transfer factors of selenium to a primary consumer through the bioconcentration and biotransformation of inorganic oxyanion species by field-collected biofilms. Samples were exposed in the laboratory to aqueous concentrations of selenite and selenate respectively, at concentrations of 0, 5 and 25 µg Se/L, and uptake by the biofilm was quantified. The amphipod *Hyalella azteca*, a primary consumer characteristic of Canadian freshwater ecosystems, grazed on the selenium-spiked biofilm communities to determine trophic transfer efficiencies as a function of community structure. Selenite was transferred in a dose-dependent fashion and final tissue selenium concentrations varied by site. *H. azteca* tissue selenium reached up to 6.47 ± 0.24 ug Se/g in the low treatment and 20.72 ± 6.06 ug Se/g in the high treatment. Trophic transfer factors from the biofilm to the invertebrate ranged from 0.16 – 0.44 in the low treatment and 0.13 – 0.34 in the high treatment. Selenate trophic transfer did not differ from control across all sites. This research will serve to assist in improving environmental risk assessment strategies for the release of selenium into aquatic environments.

**485 Effects of selenium speciation on bioaccumulation downstream of a biological water treatment plant**

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Biological treatment of selenium acts via microbial transformation of aqueous selenate (VI) to reduced species such as selenite (IV) and organoselenides (II). This process lowers aqueous total selenium concentrations, but can alter the bioavailability of the remaining selenium. We found increases in selenium bioaccumulation downstream of an active water treatment plant, despite reductions in total aqueous selenium concentrations. We combined laboratory algal uptake tests, field measurements of bioaccumulation, and selenium speciation analysis of treated effluent and receiving waters to develop a model of selenium bioaccumulation that explictly accounts for speciation. We quantitatively attributed the observed increase in bioaccumulation to reduced species, including selenite, dimethylselenoxide, and methylseleninic acid. We then applied this model to predict how bioaccumulation would be affected by a post-treatment oxidation step that returns effluent speciation to a selenate-dominated condition.

## In Situ Treatment of Contaminated Sediments – What Are the Remaining Barriers?

**486 Placement and Measurement Challenges: Methodology for Evaluating Installation and Testing of As-Placed Activated Carbon Amendments**

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Background. The successful use of activated carbon or other reactive materials for in-situ sediment treatment (via contaminant sequestration and immobilization) or the incorporation of these materials into reactive caps has been studied and demonstrated at numerous sites worldwide over many years. To determine the form and quantity of activated carbon required and the design of an in-situ treatment remedy, treatability studies have often been performed along with modeling, and assumptions are made from these studies that are important to the ultimate success of the remedy. It is important to be able to verify that the intended design was properly constructed. However, key aspects related to full-scale installation and quality control may be limiting expansion and more broad-based acceptance of this sediment remediation approach. Challenges have been experienced in quality control related to both evaluation of placement/installation and verification of as-placed activated carbon and organoclay content at the sediment surface. This presentation will focus on the means and methods successfully utilized to address these challenges. Approach/Objectives. Three aspects of the use/application of activated carbon for sediment remediation will be discussed. First, an overview of the impact of activated carbon material selection will be provided. Second, a discussion of treatability studies and data evaluations used to develop the design assumptions and the importance of confirmation of modeling and design aspects. Finally, an overview of a large-scale project will be presented to illustrate methods that have been successfully utilized to provide post-placement confirmation of installation. Aim. Field results demonstrate that the appropriate material selection and application of powdered activated carbon can provide significant reductions in pore water contaminant concentrations of PCBs and corresponding reductions in bioavailability as documented by contaminant up-take in target species. The goal is to reduce uncertainty and risk associated with remedy implementation. This presentation will provide important information related to both activated carbon selection and methods used to verify the successful placement of the material to support an In-Situ treatment approach to contaminated sediment remediation.

**487 In situ evaluation of using clean dredged material as an alternative to clean sand for enhanced monitored natural recovery**

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Portions of Pearl Harbor have been selected for remedial action under a Record of Decision due to elevated sediment concentrations for contaminants including cadmium, copper, lead, mercury, zinc, and total polychlorinated biphenyls (tPCBs). While Enhanced Monitored Natural Recovery (EMNR) has been successfully demonstrated with clean sand, the use of uncontaminated natural sediment as a cap substrate, currently termed true EMNR (tEMNR), should allow uncontaminated sediment with a natural level of binding capacity (primarily associated with organic carbon and fines content) to sequester contaminants and enhance recovery when compared with traditional EMNR. This study explored the potential value of using locally available clean dredged material in a thin layer cap, in direct comparison with other common sediment remedies, at a mesocosm scale using a series of field-deployed Remedy and Recontamination Assessment (RARA) arrays. RARA arrays were designed to provide a site-specific, direct measurement of recontamination potential and impact on a range of remedies while providing increased realism compared to laboratory treatability studies and reduced costs and complexity compared to large-scale field pilot studies. The arrays, housing multiple 18-gallon polyethylene treatment cells, were placed in an area of Pearl Harbor currently being considered for EMNR, and contain Pearl Harbor site sediment treated with a thin-layer of each of five treatments: 1) low carbon dredged material; 2) high carbon dredged material; 3) clean sand; 4) powdered activated carbon (AquaGate +PAC™); or 5) control (no treatment). The arrays incorporate standard cylindrical sediment traps to capture incoming depositional sediments, and also house an ADCP and data sondes to characterize hydrodynamic and water quality conditions, respectively, during the deployment. In addition to two monitoring events involving deployment and recovery of porewater passive sampling devices (i.e. polyethylene for PCBs and diffusive gradients in thin film (DGT) and peepers for metals) during the study, recovery of the arrays will allow for a range of endpoints including final sediment chemistry, bioaccumulation, and benthic community composition to provide the basis for evaluating effectiveness of the various remedies and potential recontamination from deposited material following a ~12-month field deployment.

**488 In Situ Passive Sampling for the Evaluation of Carbon Amendment Performance**

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In 2006, the Portland District of the U.S. Army Corps of Engineers collected sediment samples from varying depths near a portion of the Lower Columbia Slough at the Pacific Meats cleanup site. Sediment samples presented polychlorinated biphenyl (PCB) concentrations up to 2,450 µg/kg. The PCB concentrations were found to be higher in the Lower Slough than in other reaches and were detected more consistently within the more industrial and commercial development of this area. In 2015, Texas Tech University performed a baseline study by evaluating in situ and ex situ porewater sample analyses at 20 locations at two depth intervals (0-13.5 cm and 13.5-29 cm). In situ sampling was performed by solid-phase microextraction (SPME) passive sampling using polydimethylsiloxane (PDMS) coating on a glass fiber to act as a sorbent for PCBs. In 2017, a thin cap of activated carbon (AC) was placed across two separate plots (East and West). After the placement of AC in two forms, the analysis and comparison of pore water concentrations within the 20 approximately identical cap locations across the two plots was determined. SPME samplers were deployed in triplicate in the sediment for 28 days to achieve equilibrium with the contaminants of concern for both the baseline analysis and the carbon amendment results. The reductions from the baseline porewater concentrations ranged from 68-98% in the surficial sediment layer, and a central tendency of 90% reduction in porewater concentrations was presented for both the surface and deeper layer across both AC plots. Environmental drivers are presented as an indicator for the sediment porewater exchange rates and reduction of PCBs throughout the site.

**489 Evaluation of porewater reductions due to carbon placement via SedimiteTM and AquaGateTM at a contaminated sediment site**

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Contaminant flux and availability in impacted sediments can be significantly reduced with activated carbon (AC) amendment technology. AC is more often placed as a composite material to aid in settling and retention at the sediment surface. A demonstration of two such composite materials, Sedimite and Aquagate, was conducted in open water near Hunters Point Naval Shipyard (HPNS), CA. In this study, changes in freely dissolved concentration ($C_{\mathrm{free}}$) of polychlorinated biphenyls (PCBs) in the surface sediments and in deeper layers (i.e., 30 cm below the sediment surface) were evaluated by in situpassive sampling using polydimethylsiloxane (PDMS) coated fibers.Bioaccumulation reductions in bivalves exposed to AC materials was also assessed and compared to co-deployed passive samplers.*In situ*monitoring of $C_{\mathrm{free}}$with passive samplers was conducted before sediment amendment and up to 26 months following AC placement. Passive samplers with 34.5 µm PDMS coating were preloaded with a wide array of $^{13}C_{12}$PCB congeners as performance reference compounds (PRCs). PDMS samplers were inserted to unshielded holders, attached to a tripod frame and embedded vertically 30 cm into the sediment at20 sampling locations. After 28 days the fibers were retrieved, sectioned into a 1-6, 6-11, 11-16 and 21-26 cm segments below the sediment surface and analyzed for PRCs and 111 PCB congeners. Baseline sampling showed uniform $C_{\mathrm{free}}$across the site with lower concentrations in the near surface versus the deeper layers due to exchange with the overlying water from the shallower zone. Post-placement monitoring showed an 83% decrease of $C_{\mathrm{free}}$after 8 months in the surficial layer with further reductions reaching 90% after 26 months. Smaller but significant reductions in $C_{\mathrm{free}}$was also noted at the deeper depths, which continued to improve with time in line with AC mixing. Bioaccumulation of PCBs in clams (*Macoma Nasuta*) showed very good agreement with predictions from measured $C_{\mathrm{free}}$using equilibrium partition theory. The presentation provides information on the effectiveness of AC by means of $C_{\mathrm{free}}$measurements and discusses the advancement of AC technology based on results from this demonstration as well as earlier AC studies conducted at HPNS site.

**490 Assessment of In-situ Treatment of Multicomponent Contaminated Sediment using Activated Carbon under Differing Levels of Contamination and Disturbance**

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Since the first field trial of in-situ treatment using activated carbon (AC) sorbent was launched at Hunters Point Former Naval Shipyard, CA in 2004, numerous field studies followed and successfully demonstrated its effectiveness in reducing the aqueous and biological availability of various hydrophobic organic contaminants (HOCs) associated with sediments in aquatic ecosystems. Even with the demonstrated effectiveness, there is still a reluctance for regulatory agencies, responsible parties, and other stakeholders to select this technology as a full remedy. Much of this reluctance is because this relatively new and innovative remedial technology has, by definition, not yet yielded long-term monitoring results under varying site-specific conditions. For example, the performance of the AC technology under different levels of localized site disturbances (e.g. potential scour by vessels) and on-going contaminant influx have limited long-term results in the field. To help address this perceived gap, we proposed a pilot study at the former United Heckathorn site at Lauritzen

Channel in Richmond, California, where sediments were impacted by DDT and various co-contaminants. Successful laboratory tests showed the benefit of about 4% AC dose in treating DDT levels ranging from several ppm to 2000 ppm. In the field study, we will assess the application of AC as a blended cover mixed with sand/or gravel. We will assess the blended cover's performance, stability, and other remedial benefits. The blended cover is specifically designed to increase the material layer's resistance to erosion, accommodate localized turbulence, promote helpful mixing of AC with sediment, and limit scour depths. This pilot study will evaluate the feasibility of the in-situ AC technology under various site conditions: localized hydrodynamic disturbance, varying contaminant sediment concentrations, and on-going contaminant influx. This presentation will focus on the pilot study design and preliminary treatability study results.

**491 A novel, activated carbon-based material for the in situ remediation of contaminated sediments**

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Using activated carbon (AC) for the in-situ treatment of contaminated sediment has been established as a promising alternative to traditional remediation methods. However, a recent field test in Lake Kernaalanjärvi (Finland) has indicated that this cost-effective remediation method can have significant shortcomings. The major problem encountered was an insufficient stability of the applied AC thin layer cap. The high losses of the capping material were caused by turbulent water conditions due to the large surface area and shallow depth of the lake and the fine particle size of the applied AC (< 100 µm). While these powdered ACs (PAC) have been found to achieve the highest contaminant binding efficiency, their low bulk density facilitates their unintended transport. In addition, they can cause stronger adverse effects to organisms than granular ACs (GAC). However, the latter was found to bind the PCBs from the lake's sediment insufficiently. Therefore the development of a novel remediation material was initiated. To achieve an improved stability in turbulent waters and high remediation efficiency, PAC and clay were fused together under high heat to form stable aggregates. $NaHCO_3$ was added to increase porosity. In the resulting, sandstone-like material, a large share of the PAC's surface remains exposed to the surrounding matrix. The larger overall particle size and added bulk of the aggregates aim at an improved resistance against water movements and lowered adverse effects. Furthermore, the material sinks rapidly through the water column, which eases its amendment and reduces losses due to drift from the intended application site. The aggregates were produced with a grain size of 400 – 1000 µm and compared to GAC of the same particle size. Doses of 0.75, 2, 4 and 7.5 % (based on AC content and sediment dry weight) were applied to natural, PCB-contaminated sediment. *Lumbriculus variegatus* and *Chironomus riparius* larvae were used as test organisms. The novel material showed significantly higher remediation efficiency than the GAC in similar doses, with a reduction in PCB-bioaccumulation of up to 93% (*L. variegatus*) and 82% (*C. riparius*). Significant adverse effects of the novel material were not observed for *C. riparius* and only for 4 and 7.5 % doses for *L. variegatus*. Although at an early point in development, the AC-clay aggregates have shown a promising potential as an alternative in environments with unsuitable conditions for other AC-based materials.

**492 The challenge of measuring activated carbon dose in sediments**

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The use of activated carbon (AC) for sediment remediation has increased in acceptance and application for in-situ remediation of persistent organic pollutants (POPs) and hydrophobic organic compounds (HOCs). In-situ application has demonstrated that an addition of 1-5% black carbon material can significantly reduce bio-uptake into aquatic food webs. The most important factor in the success of the technology is the dose and uniformity of AC that is actually delivered and persists in sediments post application. However, there is no standard method to measure the amount of AC in sediment, and researchers have used a variety of techniques including measures of total organic carbon, loss on ignition, traditional black carbon determination, and a specialized method for AC. In this work, two different carbon treatments (AC and Biochar) were measured for the amount of black carbon after 7 years of implementation in a field pilot study. Three analytical methods (loss on ignition, total organic carbon, and specialized AC measurement) were compared for the efficacy of measuring the amount of black carbon present in the sediments. A comparison of the accuracy and precision of each method and recommendation for adoption is presented in this study. Accurate measurement of AC in sediments as demonstrated in this study is necessary to build confidence in the adoption of in-situ remediation of sediments.

**493 Assessing the Effects of Activated Carbon-based Amendments on the Bioavailability of Methylmercury from Marsh Sediments to Aquatic Invertebrates**

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Studies were conducted to evaluate the effect of activated carbon (AC) amendments on the bioaccumulation of methylmercury (MeHg) in marsh sediments to *Leptocheirus plumulosus*, a representative benthic test organism. In one study, effects of two types of AC amendments—powdered AC and SediMite$^{TM}$ (a pelletized agglomerate of powdered AC)—were compared to an unamended control sediment. In another study, the effects of powdered AC in a newly amended sediment and a powdered AC-amended sediment aged under field conditions for 20 months were compared to an unamended control. Concentrations of MeHg in porewater were also examined as a measure of bioavailability. Samples of surficial marsh sediment were homogenized and sieved to remove plant material. Sediment from each treatment was allocated to replicate test beakers, and redox conditions were re-established before adding test organisms. Samples were collected periodically for analysis of sediment (MeHg and sediment organic carbon), porewater (MeHg), and tissues (MeHg and lipids). Redox conditions and pH were monitored continuously. Several prior tests were performed to develop a method that would preserve MeHg concentrations in test soils during the exposure period. Soil and porewater Hg, MeHg and related geochemistry were tracked carefully prior to and during exposure in both studies. In both experiments, concentrations of MeHg in porewater were significantly lower in all AC treatments compared to the unamended control. Larger reductions in porewater concentrations in comparison to the unamended control were observed in the freshly amended AC treatment (up to 50-fold) than in the aged AC treatment (up to 4-fold). Two to three-fold reductions in tissue concentrations were observed in the freshly amended AC treatments and significant differences between freshly amended AC treatments and unamended controls were observed on Day 7 and 14 in one study, and on Day 21 in the other study. Concentrations of MeHg in tissue from the aged AC treatment were significantly higher than in the unamended control, possibly reflecting significant increases in MeHg in the aged AC treated sediment over the course of the experiment. After normalizing tissue concentrations to sediment concentrations (i.e., accounting for differences in sediment MeHg concentrations) results indicate that both fresh and aged AC decreased bioavailability of MeHg (up to ~3- to 4-fold) in comparison to the unamended sediment.

## Developments in Water Quality Monitoring and Analytical Methods in Support of Water Reuse – Part 2

### 494 Non-target Characterization of Endocrine Active Mixtures Impacting Minnesota Sunfish Spawning Habitats

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Environmental trace organic contaminant (TOrC) mixtures are suspected to have additive or synergistic adverse effects on wildlife. The objective of this study was to identify components of aqueous TOrC mixtures at Minnesota sunfish spawning habitats that may contribute to the endocrine disruption of resident fish. Porewater samples from four sunfish spawning habitats within the same lake were analyzed using high-resolution mass spectrometry (HRMS). Blood vitellogenin concentrations from captured adult male bluegill sunfish served as biological endpoints of estrogen agonism to bin sites as either Active or Inactive. We hypothesized sites with more pronounced estrogen agonism (Active) would share unique features at higher intensities relative to Inactive sites. Non-target features of interest were prioritized according to multivariate (Principal Component Analysis; PCA and Principal Component Variable Grouping; PCVG) and univariate (two-tailed t-test) analyses. Active and Inactive samples showed unique clustering patterns within PCA scores plots. Corresponding features in visually notable PCVG groupings with significantly (t-value > t-critical) higher intensities in all Active replicates were prioritized for identification. Reported features were identified using existing spectral libraries or in silico fragmentation databases and ranked according to established confidence standards.

### 495 Not My Fish: Using acoustic telemetry to explain contaminant variation in sentinel species along the OCSD outfall

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To abide by permit requirements approved by the EPA and RWQCB, ocean monitoring staff at the Orange County Sanitation District (OCSD) regularly monitor tissue contaminant levels (e.g., PCBs and DDTs) in demersal fishes that are locally abundant. Results have shown high contaminant variation both within and among species across sampling sites located ~8 km apart. Variation in contaminant levels may be explained by differences in inter- and intra-species movement patterns, resulting in some individuals experiencing different levels of contaminant exposure. In this study, acoustic telemetry (Vemco Positioning System) is used to gather data on the movement patterns of tagged individuals that can be used to determine which species are consistently exposed to contaminants from the OCSD outfall and are thus appropriate indicator species of its wastewater effluent. One hundred fifty fish in the orders Pleuronectiformes (flatfishes) and Scorpaeniformes (scorpionfishes and rockfishes) were tagged in regions surrounding the outfall pipe and at a nearby reference site (~8 km upcoast) between March and June 2017. Preliminary results suggest that pleuronectiform fishes are highly mobile and capable of moving up to 8 km in less than 10 hours. These fishes appear to cover large areas that change daily (95% Brownian Bridge KUD: 0.043 ± 0.039 $km^2$ (n=46) at the outfall site; 0.178 ± 0.271 $km^2$ (n=17) at the reference site), and do not appear to show a long-term association to the outfall pipe, as most individuals left the array within two months of tagging. Scorpaeniform fishes, on the other hand, remained within small areas (95% Brownian Bridge KUD: 0.011 ± 0.015 $km^2$ (n=53) at the outfall site; 0.0003 ± 0.0004 $km^2$ (n=3) at the reference site) near the outfall diffuser region for several months. These data indicate that scorpaeniform fishes are more appropriate indicators of wastewater effluent effects than pleuronectiform fishes, as they are more likely to be chronically exposed to contaminants surrounding the outfall. The results further demonstrate that acoustic telemetry is a useful tool for selecting appropriate indicator species for monitoring purposes and assuring that comparisons of tissue contaminant data of sentinel species are scientifically defensible.

### 496 Human Exposure Assessment and Relative Source Contribution of Phthalates and its Metabolites in China's Drinking Water

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Phthalates are endocrine disruptors that are commonly found in drinking water and have received wide attention due to their high yields and reproductive developmental toxicity, simultaneously, phthalates monoesters have been recognized as the bioactive metabolites, which could elicit stronger reproductive toxicity than their parent compounds. As drinking water is a major exposure pathway of contaminant, it is crucial to investigate the concentrations and distributions of phthalates and monoesters in drinking water, estimate human exposure of these compounds, and assess their relative source contributions (RSCs) of drinking water. However, there was little paper to systematically calculate contributions for phthalates and monoesters in drinking water. In this study, phthalates and their metabolites were frequently detected in drinking water from 100 drinking water treatment plants of 24 cities in China. The mean concentrations for DMP, DEP, DiBP, DnBP and DEHP in drinking water were 74.9 ± 516, 22.1 ± 42.6, 232 ± 787, 473 ± 1349 and 148 ± 258 ng/L, respectively. The primary metabolites, MMP, MEP, MiBP, MnBP and MEHP, and secondary metabolites of DEHP, MEHHP, MEOHP, and MECPP were for the first time detected in drinking water with the mean concentration of 12.4 ± 18.5, 2.3 ± 6.0, 11.3 ± 38.3, 37.3 ± 113, 9.4 ± 18.1, 0.25 ± 0.49, 0.15 ± 0.34 and 0.12 ± 0.38 ng/L, respectively. Geometric mean concentrations of urinary MMP, MEP, MiBP, MnBP, MEHP, MEOHP, MEHHP and MECPP were 10.1, 19.3, 29.6, 47.3, 3.6, 6.0, 11.6, 14.4 μg/g creatinine, respectively, in 1040 participants of 16 cities of China, thus the total daily intakes (TDIs) of DMP, DEP, DiBP, DnBP, DEHP for Chinese population were calculated to be 0.32, 0.64, 1.05, 1.41, and 1.33 μg/kg bw/day, respectively. The RSC of DEHP in drinking water was 0.52%, which was lower than 1% suggested by WHO, and of DMP, DEP, DiBP and DnBP, RSCs were 0.05%, 0.03%, 0.36% and 0.26%, respectively. In addition, the contributions of MMP, MEP, MiBP and MnBP in drinking water were 0.14%, 0.02%, 0.05% and 0.10%, respectively. This was the first time to obtain the RSCs of phthalate metabolites in drinking water.

### 497 Surface and groundwater pollution with municipal waste in a water scarce metropolis: A study of Lagos State, Nigeria

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The use of dumpsite instead of landfill systems for municipal waste disposal is a common method in many developing countries which is usually far from recommended standards. Dumpsite are sources of surface and groundwater pollution due to the production of leachate and its migration through refuse. Municipal Solid Waste (MSW) disposal within the Lagos metropolis is of concern as poverty, population growth and high urbanization rates combined with ineffective and under-funded municipalities to prevent the efficient management of wastes. This study assessed the concentration of Poly Brominated di-Phenyl Ether (PBDEs) in leachate and water from four active and one abandoned dumpsites within the Lagos metropolis using standard methods and analysed using GC-MS. The mean concentrations ranged from 7.11±10.1 (Ikorodu) to77.5±109.6 $\mu gL^{-1}$

(Abule Egba) for BDE 28; 5.37±0.3 (Olusosun) to 31.5±27.3μg$L^{-1}$ (Abule Egba) for BDE 47; 17.01±24.1(Abule Egba) to183.1±225.7 μg$L^{-1}$ for BDE 100; 11.7±16.5 (Abule Egba) to173.5±216.5 μg$L^{-1}$(Epe) for BDE 99; 117.4±166 (Abule Egba) to 2033.5±2819.4 μg$L^{-1}$(Epe) for BDE 183; 295.8±391.6 (Ikorodu) to 4282.6±1277.5 μg$L^{-1}$ (Epe) for BDE 209. Olusosun, Solous. Abule Egba had the highest concentrations of BDEs 28 and 47 while Epe dumpsite had the highest concentrations of BDEs 100, 99, 183 and 209. Figure 2 shows the mean concentrations of PBDEs in leachate samples from the five dumpsites in Lagos state. BDEs 28 and 153 were not detected in all the leachate samples from the five sites. BDE 154 was only detected at leachate of Olusosun dumpsite. The mean concentrations ranged from 5.80±1.94 (Epe) to 16.9±9.13 μg$L^{-1}$ (Abule Egba) for BDE 47; 3.9±5.95 (Abule Egba) to 119.3±149.9 μg$L^{-1}$ (Ikorodu) for BDE 100; 1.4±1.98 (Abule Egba) to 105.2±140.4 μg$L^{-1}$ (Ikorodu) for BDE 99; 17.5±24.8 (Abule Egba) to 942±1298.7 μg$L^{-1}$ (Ikorodu) for BDE 183 and 35.7±18.7 (Abule Egba) to 7775±7036.6 μg$L^{-1}$ (Olusosun) for BDE 209. BDE 47 had the highest concentration at Abule Egba dumpsite while its least concentration was recorded at Epe dumpsite. In all the five sites, Olusosun dumpsite leachate had the highest $?_8$PBDES of 8470.35μg$L^{-1}$ It can be concluded from the study that municipal solid waste contributes significantly to PBDEs pollution of surface and groundwater pollution within the Lagos metropolis. Also, there is urgent need to put in place remediation programmes to clean contaminated water to prevent future outbreak of water borne diseases within the metropolis.

**498 Effects of NPs (TiO2) in Wastewater on the Plant Uptake of Contaminants**

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Freshwater demand for irrigation is increasing with the food demand of the growing global population. At the same time, industrial development and the expanding urbanization are also demanding more freshwater, resulting in an increasing volume of wastewaters (WW). Many countries are facing the problem of freshwater scarcity and wastewater disposal. As a result, farmers in several developing countries are using, knowingly or unknowingly, partially treated and/or untreated WW to irrigate crops. The presence of contaminants including heavy metals, hormones, pharmaceuticals and personal care products in WW are harmful for environment and human health. In addition, titanium dioxide nanoparticles (NPs-$TiO_2$) have also been detected recently in WW; and their interaction with contaminants in soil-water systems is unknown. $TiO_2$ could interact with WW contaminants and reduce their mobility and uptake by plants. A field lysimeter study has been conducted for two years (2017 and 2018) to investigate the effect of $TiO_2$ on the mobility of heavy metals to potatoes. Potatoes were planted in a sandy soil and irrigated with WW supplied with/out NPs. The WW itself contain organic contaminants and heavy metals (Cd, Zn, Cu, Fe, Pb, and Cr). The edible parts of potato plant (flesh and peels) are used to examine the presence of heavy metals levels using ICP-MS. Results for the first season showed that the presence of NPs in WW reduced lead level in potato peel to 0.2 μg/g as compared to 0.5 μg/g in control (WW without NPs); NPs did not affect the translocation of lead to the flesh of potato (0.24 μg/g in both the treatments). In potato peels, NPs in WW reduced cadmium level to 1.5 μg/g as compared to 11 μg/g in WW without NPs; the corresponding concentrations in potato flesh were 0.45 μg/g and 2.0 μg/g. From the early studies it seems, generally, heavy metals were found to be retained in soil when nanoparticle was added to the wastewater. The results of second year would confirm the findings.

**499 Study on the heavy metal adsorption characteristic of graphene oxide-based calcium alginate bead and its magnetic bead**

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At present, water pollution caused by heavy metal ions is still a big problem and has become a serious threat to human and aquatic lives. Therefore, special concern must be paid to the treatment of heavy metal ions. In our research, graphene oxide-based calcium alginate beads and its magnetic beads were prepared by using the advantages of graphene oxide and sodium alginate to adsorb heavy metal cations. They were prepared by using several raw materials such as sodium alginate, calcium chloride, ferric chloride, ferrous chloride and graphene oxide which was prepared by Hummers' method. Then they were characterized by FT-IR, SEM, and XRD. The SEM result shows that the two kinds of beads are spherical in shape, and their surfaces are wrinkled while their interior structures are faveolate. These could greatly increase the specific surface areas with the active sites for adsorbing heavy metal ions. The X-ray diffraction results show that the molecular chains of sodium alginate intercalate into graphene oxide layer, which forms the interlayer structure, resulting in the further increase of the gap between the layers of graphene oxide. The two kinds of beads were applied to absorb the copper ions and cadmium ions in aqueous solutions, and the magnetic graphene oxide-based calcium alginate bead was also applied to the adsorption of cadmium ions in real food samples of oyster homogenate. Their adsorption characteristics were been studied and fitted with kinetic models, and the amount of the beads, the adsorption time, pH values and other conditions were been optimized. All the research data indicated that these beads had achieved very good results in the adsorption of such heavy metal ions, with the adsorption rates of 91.1% to 93.3%.

**500 NH3 vapour induced -NH2 deprotonation at gas-liquid and gas-solid interfaces: A novel step for analytical devices development for environment**

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Molecules containing polarized N-H bonds are extensively being used as receptors for charger analytes (*usually anions*) in aprotic solvents. The ubiquitously found receptors typically utilize N-H fragments, for hydrogen bonding or proton transfer between donor (receptor) and acceptor (anion). Even though such recognition platforms have been comprehensively studied, but it is important to highlight that their sensing towards gaseous analytes are unheard of. Receptors that can respond upon interface with analyte vapours (*gas-liquid interface between receptor and analyte vapours*), could be highly valuable for forensic, environmental, industrial and explosive screening. As polarized N-H receptors are selective, development of specific receptors in this direction will be highly rewarding. Additionally, accomplishing such performances in solid phase of receptor (gas-solid interface between receptor and analyte vapours) will offer a pivotal-step towards the development of convenient and portable devices for analyte sensing in ambient air. In this work, we demonstrate an easy to synthesize (one-minute-synthesis) –$NH_2$ receptor, from diaminomalenonitrile (DAMN). The dye exhibit vapour induced -NH-H deprotonation from highly electron deficient –$NH_2$ group. For the first time, such vapour induced –NH-H interaction endorses unprecedented charge transfer (CT) across the system (*red-shift of 166 nm*). The mechanistic insight was further developed for specific ammonia vapour sensing under room temperature, *via* chromogenic readout. The operation of proton transfer signaling mechanism between receptor and vapor allocates it to completely regain original state of the molecule*via* control inputs in the form of acid vapour. Most importantly, such events were realized in the solid support of dye, impregnated on a silica surface without an chemical anchoring, signifying a pivotal-step towards development of convenient, cheap and portable analytical technology. Thus inclusion of such a receptor strategy in molecular recognition chemistry, alarms potential applications for chemical apportionment of gases in ambient air, unlikely of the existing receptors.

## Fate and Effects of Chemicals from Diffuse Sources and Stormwater – Part 2

### 501 Using passive sampling to assess PAH bioavailability from stormwater-associated sediment recontamination

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Urban stormwater runoff has long been identified as a major influence to the contamination of receiving water bodies and sediment. The episodic nature of storms combined with the imperviousness of urban surfaces, lead to stormwater discharges laden with high levels of solids-associated Priority Organic Pollutants (POPs). Benthic communities are one of the main receivers of such particles and are accumulators of POPs. The objective of this study was to assess the physicochemical characteristics of Polycyclic Aromatic Hydrocarbons (PAHs) found in storm runoff solids and their bioavailability to the benthos. Samples were taken during the 2015/16 and 2016/17 storm seasons at Paleta Creek at Naval Base San Diego (NBSD). The effects of the storm seasons on receiving waters were assessed by size fractioned stormwater sampling, sediment traps and cores, porewater passive samplers and in situ bioaccumulation studies with bent-nose clams (*Macoma Nasuta*) with a Sediment Ecotoxicity Assessment Ring (SEA Ring). Ex situ passive sampling and bioassays were also performed on dry weather and wet weather sediments along with additional dry weather sediment cores spiked with accumulated trap material. The stormwater sampling showed much of the PAHs were associated with large particles in runoff and led to rapid deposition and sediment recontamination in receiving waters. The bioavailability of that sediment recontamination, however, was limited as indicated by bioaccumulation in organisms and passive sampling measurements of porewater concentrations. Porewater concentrations predicted bioaccumulation in in situ bioassays with an effective bioaccumulation factor approximately equal to the octanol-water partition coefficient. Ex situ bioassays were also predicted by porewater concentration measurements if sediment trap solids were added to bioassays, suggesting the importance of stormwater deposited solids on benthic exposures and the potential misleading effects of homogenization of surficial sediments in ex situ bioassays. Sediment concentrations were not a good indicator of bioaccumulation with BSAF values

### 502 Pavement sealants--A diffuse source of PAHs and related chemicals to stormwater

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Pavement sealants are widely used throughout North America to protect and beautify the asphalt pavement of driveways and parking lots. Diffuse releases of chemicals associated with coal-tar-based (CT) pavement sealant—the type commonly applied to pavement in the central, southern, and northeastern U.S. and in Canada—have implications for aquatic-ecosystem health. CT sealcoat typically is 20 to 35% crude coal tar or coal-tar pitch and contains on the order of 50,000 to 100,000 mg/kg PAHs and related compounds, about 1,000 times more than alternative pavement sealant products. Since the early 2000s, research by government agencies, academic institutions, and non-governmental organizations has advanced understanding of the importance of this diffuse source of chemicals to stormwater quality and aquatic ecosystems. Research has included evaluation of rates of application, stormwater loading, air emissions, and physical wear; transport in both solid and whole-water forms; chemical profile evolution; and phototoxic effects. This presentation will synthesize the existing knowledge on CT pavement sealants and their relation to stormwater and stream contamination.

### 503 Primary sources of PAHs to Great Lakes tributaries using a multiple-lines-of-evidence approach

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Polycyclic aromatic hydrocarbons (PAHs) are among the most widespread and potentially toxic contaminants in Great Lakes tributaries. Urban sources of PAHs are numerous and diverse, and identifying the primary source(s) to aquatic environments can be difficult. This study used a multiple-lines-of-evidence approach to determine the likely primary source(s) of PAHs in sediment samples from 71 locations across 26 Great Lakes Basin watersheds. By using a multiple-lines-of-evidence approach, the uncertainties of each method are mitigated and common conclusions are strengthened. Diagnostic ratios, 11-compound profiles, and principal components analysis indicated that coal combustion, vehicle emissions, and coal-tar pavement sealant were the most likely primary sources of PAHs in a majority of sediment samples. Of those, coal-tar pavement sealant was found to be the most likely primary source based on mass fractions analysis. Results from land-use analysis and Positive Matrix Factorization source-receptor modeling are pending. In addition to PAH source identification, the potential toxicity to aquatic organisms was assessed by comparison to established sediment quality guidelines. The Threshold Effect Concentration was exceeded at 62% of sampling locations, and the Probable Effect Concentration and (or) the Equilibrium Partitioning Sediment Benchmark were exceeded at 41% of sampling locations.

### 504 Forensic analysis of polychlorinated biphenyls in wastewater in the Mid-Atlantic region of the USA

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Polychlorinated biphenyls (PCBs) are persistent, toxic and bioaccumulative pollutants. One of the few pathways via which they break down is microbial dechlorination, which has been shown to occur in sewers. Questions remain about where within sewers this process takes place and which conditions encourage dechlorination. These issues were examined using a large data set on PCBs in influent and effluents from a main and bypass outfall from a wastewater treatment facility in the Mid-Atlantic region of the USA. A data set containing 64 chromatographic peaks representing 94 PCB congeners measured in 74 whole water samples was analyzed by Positive Matrix Factorization (PMF). PMF resolved four factors and three factors represented Aroclors 1242, 1254, and 1260. The last factor represented an advanced dechlorination regime of PCBs characterized by high proportions of PCBs 4 and 19. Concentrations of the four factors were regressed against various parameters. Concentrations of dechlorination products were not correlated with total suspended solids, indicating they were mostly dissolved explaining their poor removal during the treatment process. Even though treatment generally reduces the dioxin-like toxicity of the PCB mixture, this effect might be offset by the incomplete removal of dechlorination products. Concentrations of dechlorination products were also not correlated with flow, suggesting that instead of occurring in sediment deposits that are readily scoured at high flow, dechlorination likely occurred in cohesive sediments, which have long residence times, and time to develop anaerobic conditions and harbor a slow process such as dechlorination.

**505 A Statistical Assessment of Factors Influencing PCB Concentrations Observed in Stormwater in the San Francisco Bay Area, California**

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The San Francisco Bay polychlorinated biphenyls (PCBs) total maximum daily load (TMDL) implementation plan calls for control measures to reduce PCB loads entering the Bay via stormwater. To support this plan, a stormwater screening monitoring program was implemented in water years (WYs) 2011, 2015, 2016, and 2017 at 71 sampling sites. At each of these sites one storm composite sample was collected. The number of samples is much smaller than the number of factors (hydrological and climatic factors as well as watershed characteristics) that can potentially influence the observed PCB concentrations and loads. In addition, there exist significant correlations among some of the factors, and the relationship between the PCB concentrations and the factors are not necessarily linear. Facing these challenges, this study developed a staged statistical procedure, utilizing methods such as partial least square regression and random forests model, to identify the factors most influencing PCB concentrations observed in stormwater. The most influential factors will be presented. These factors can be used to build mechanistic models of pollutant transport and fate, or/and rank the pollution potential of watersheds where no or few samples exist currently, allowing prioritization of areas to focus more monitoring and management effort in the future.

**506 Regional Monitoring of Biological Condition in San Francisco Bay Area Streams**

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In 2010, the Bay Area Stormwater Management Agencies Association (BASMAA) Regional Monitoring Coalition (RMC) embarked on a regional monitoring effort to evaluate the biological condition of wadeable streams in the San Francisco Bay region. Stream bioassessment monitoring was coordinated among six Bay Area municipal stormwater programs that conducted monitoring using standardized sampling protocols. Biological condition was assessed using four primary indicators: benthic macroinvertebrates (BMI), soft algae, diatoms, and physical habitat. During the first five years of RMC bioassessment monitoring (2012-2016), 354 randomly selected sites were sampled, representing both urban (81% of sites) and non-urban (19% of sites) land uses. The distribution of BMI and algae index scores suggests the majority of streams in the RMC sample area do not currently support healthy biological condition, with the majority of poor scores derived from urban sites. Overall, over two-thirds of the sites exhibited California Stream Condition Index (CSCI) scores in the Very Likely Altered condition class (< 0.63), while less than 10% of sites were in the Likely Intact condition class (> 0.92). Notably, both of the diatom and soft algae index scores (D18 and S2) exhibited higher condition scores than CSCI across the region. This may suggest that Bay Area streams are less degraded for algae communities, than for BMI assemblages. Random Forest (RF) models were developed to evaluate stressors associated with biological condition scores within the RMC area. The RF model of CSCI scores indicated that land use (% imperviousness) and physical habitat (% fines, % gravel, % riffle) were the most influential variables on BMI condition scores. In contrast, the RF model for D18 indicated water quality variables (conductivity, phosphorus, chloride) and physical habitat (% fines), exerted most influence on algae condition. The regional dataset generated through the collaborative program has aided stormwater managers in evaluating the ecological condition of Bay Area streams in a consistent manner, and prioritize efforts to assess how impacts can be mitigated.

**507 Biosolids-derived Dissolved Organic Matter Quality and Impact on Antimicrobial Bioavailability**

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Final wastewater treatment biosolids are a widely used agricultural soil amendment because they increase nutrient concentrations of soils. Although the land-application of biosolids is a means of recycling, halogenated antimicrobials within biosolids can impact crops and terrestrial and aquatic ecosystems. Environmental dissolved organic matter (DOM) has been shown to impact the bioavailability and transport of organic compounds, such as pesticides, by enhancing solubility from solid soil matrix. While it is well-established that DOM influences the fate of organic chemicals from agricultural lands, limited work has characterized emerging contaminant interactions with DOM derived from biosolids. Furthermore, little is known about biosolids-derived DOM as a function of wastewater solids stabilization processes, including (i) anaerobically digested, (ii) aerobically digested, and (iii) limed biosolids. This work characterizes DOM from different solid-stabilization processes and probes chemical interactions with halogenated antimicrobials such as triclocarban (TCC), triclosan (TCS), and degradation product and herbicide 2,4-dichlorophenol (2,4-DCP). Biosolids-DOM has been extracted from biosolids collected from nine wastewater treatment facilities, with three facilities per stabilization method. DOM characterization by fluorescence spectroscopy revealed shifts from microbial by-product-like fluorophores in limed biosolids-DOM to greater fulvic acid or humic acid-like content in DOM derived from digested biosolids. Furthermore, high-performance size-exclusion chromatography revealed the presence of large molecular weight fractions of 1300 Da to >15,000 Da in anaerobically and aerobically digested biosolids-DOM. Limed biosolids-DOM consisted of smaller molecular weight fractions, suggesting that digestion processes could be increasing the heterogeneity of DOM. Fluorescence spectroscopy is also being applied in quenching experiments with DOM-contaminant solutions. After adding contaminant TCC and 2,4-DCP, quenched fluorescent DOM regions exhibit contrasting, pH-dependent interactions with different biosolids-DOM. Lastly, we will present results of a bacterial luminescence test applied to screen toxicity and bioavailability of TCC, TCS, and 2,4-DCP with biosolids-DOM solutions. This work evaluates molecular-scale dynamics pertinent to the diffuse release of emerging contaminants derived from biosolids application.

**508 Diffuse Sources of Fecal Indicator Bacteria in Stormwater: Does it make you sick and where is it coming from?**

*K.C. Schiff, Southern California Coastal Water Res.*

Fecal indicator bacteria (FIB) are amongst the most ubiquitous and challenging types of pollutants in urban and non-urban stormwater runoff. In California alone, there are more than 320 waterbodies with a total maximum daily load (TMDL) for FIB. FIB include microbes such as *Enterococcus* or fecal coliforms, which are not pathogens themselves, but co-occur with pathogens in sewage that can make you sick. However, FIB can also come from any warm-blooded animal (birds, wildlife, etc) confounding studies to track sources for remediation or assessing human health risk. This six-year study addresses three major management gaps in San Diego, California, a county with more than 100 million beachgoers annually (including during the rainy season), and is facing regulatory enforcement for wet weather FIB. The first management gap was an assessment of beachgoer health and a quantitative epidemiology study to measure human health risk. Surveys encompassing more than 10,000 beachgoer days were used to assess the risk of 12 different symptoms

including highly credible gastrointestinal illness. Second, more than 24 stormwater discharge samples to swimming beaches across six different wet weather events were collected and analyzed for FIB, human-specific genetic source tracking markers (HF183), and 8 different human pathogens including viruses (e.g., Norovirus I and II). Third, upstream source tracking in the adjacent urban watershed was conducted to identify the source of the human specific markers and pathogens. Twenty-six wet weather site-events were collected along the mainstem and major tributaries of the San Diego River to identify if human fecal pollution was arising from a single large source (i.e., sanitary sewer overflow, illicit connection, etc) or systemic arising from multiple locations throughout the watershed, either of which would require very different management approaches and remediation strategies.

## Effective Science Communication: Case Studies and Lessons Learned

### 509 Doumont's Three Laws of Effective Communication

*A. Goldberg Day, ARCADIS*

Jean-Luc Doumont, a recognized expert in scientific presentations, describes three general rules for effective communication. These include adapting the presentation to the audience, using a succinct message and repeating that message. He discusses how the main message needs to provide added value to the audience so they will engage with the presentation. "A message interprets the information for a specific audience and for a specific purpose (Jean-Luc Doumont)." This talk will provide details on implementing Doumont's three laws. This will include suggestions on how to adapt to your audience and take responsibility for the success of the communication. Also, in an effort for a concise presentation, examples will be given on how to maximize the signal to noise ratio, where noise is anything that could distract the audience from the message. Examples of "noise" include: speaker using repetitive gestures or sayings, excess animation on slides, laser pointers, and inconsistencies on slide. A discussion on a few common problems will also be presented. Some of these common problems include slides that are prepared for the speaker instead of the audience and slide that provide too much information, such as copy and pasting from a report. Also, in an effort to save time, slides from previous presentations can be put together without an overall cohesive objective. The goal of the presentation is to provide tools to make us all better at public speaking. The audience members should be able to take away three simple ideas that can lead to more effective presentations: easy to remember, readily applicable, and always relevant. The presentation will provide examples of messages and slides that work and ones that don't. Additionally, examples will be given on how the same message should be presented differently for two unique audiences.

### 510 A Communication Strategy to Limit the Use of Flame Retardants, PFAS, and other Chemicals Classes of Concern

*A. Blum, T. Bruton, Green Science Policy Institute*

Since 2011, the Green Science Policy Institute has developed a joint strategy with academic and NGO collaborators to more effectively communicate our peer-reviewed scientific papers. We design policy-relevant research projects and use our communication strategy to bring our scientific research results to a large audience of decision makers. Our research and communication strategy has contributed to our papers having high impact scores and many thousands of downloads. Wide communication of our papers contributed to changes in policy and purchasing for reduced use of harmful chemicals such as flame retardants and highly fluorinated chemicals or PFAS. Examples include: (1) Our 2011 and 2012 studies finding flame retardants in juvenile products and furniture, which contributed to the 2013 update of California's furniture flammability standard that increased fire safety and reduced the need for flame retardants in these products across the US. (2) Our 2015 Madrid Statement documenting a scientific consensus on the need to reduce the use of PFAS, which brought this issue to the media and a broad audience. The statement provided a scientific bases for some large consumer product manufacturers and retailers to remove PFAS from the products they manufacture and sell. (3) Our 2016 paper analyzing sources of PFAS contamination in U.S. drinking water, which has contributed to remediation and policy change. (4) Our 2017 paper on PFAS in food contact materials, which educated the supply chain and has contributed to policy change and a decreased use of PFAS. Here is an overview of our communications strategy which we will discuss in more detail: Select a research topic to support policy in public interest. Collaborate with expert authors from multiple institutions. Once a manuscript is accepted, work with the journal to select a release date several weeks in the future for maximum impact. Compose a press release in accessible language with a "hook" to attract attention. Query journalists and then share the press release and embargoed paper. Educate journalists on the science and establish relationships. We encourage other scientists to consider such a communications strategy to increase the impact of their research publications.

### 511 The potential and challenges of photography in science communication

*A. Bonisoli Alquati, California State Polytechnic University, Pomona / Department of Biological Sciences*

Communication through images is arguably more prevalent than ever in our culture, largely thanks to its relatability and prevalence in social media. Journalism, including environmental journalism, has been increasingly harnessing the power of images to illustrate news and create engagement. On the contrary, the use of images in science communication by scientists is only in its infancy. I argue here that photography provides a powerful tool for scientists to effectively communicate their research. This is particularly true in the most recent phase of science communication, which is defined in terms of public engagement, rather than as a tool for spreading scientific literacy. Photographs can also help scientists solve some of the challenges that they typically face when involved in science communication, precisely because of their training. Many scientists find it difficult to summarize their findings without relying on extensive details and on the use of technical jargon, two common expressions of the so-called 'curse of too much knowledge'. Visual communication provides a way out of such difficulties, being relatable, memorable and concise – all of which are features praised by science communicators as effective ways to engage non-scientists. Photographs can also be distributed without modification, allowing a message to faithfully spread, and can be combined to narrate stories and convey powerful messages. Yet, as promising as all of this sounds, the use of photography in science communication comes with its own challenges. For example, contrary to the common concept of photography as an objective way of portraying reality, photographs are the result of technical and semantic choices, which reflect the subject taking them. The use of images in science communication requires more than the ability to craft images – it entails thinking in terms of the meaning and implications of those images. In addition, an effective use of photography in science communication also requires scientists to become adept at storytelling through images, which comes with its own series of rules and challenges. All scientists can learn those rules, but there is a long road ahead.

### 512 ScienceBites: A Multidisciplinary Online Community for Early-Career Scientist Writers

*C.A. McDonough, Colorado School of Mines / Civil and Environmental Eng*

The "Bites" family of blogs (astrobites.org, oceanbites.org, envirobites.org, chembites.org, and many others) provide a platform to connect early-career scientists with non-expert audiences by sharing compelling, bite-sized research summaries in a wide variety of scientific fields. Until recently, the Bites Sites have existed as an ad-hoc collective, with each site run independently by rotating teams of graduate-student and early-career scientist volunteers. ScienceBites is a new collaborative network run by representatives from each of these sites. We are bringing the "Bites Sites" together to work toward our common goals and increase visibility of all of the different Bites Sites among our readership. Early career scientists (graduate students and postdoctoral researchers) in STEM fields are an untapped source of very enthusiastic science writers, eager to share their research stories with the broader public. However, they often lack confidence in communicating with broader audiences, and they may have difficulty finding training resources to guide them in honing messages for non-experts, or platforms where they can publish their non-scientific writing. At the ScienceBites blogs, these scientist writers regularly publish articles translating recent research articles in their field into engaging blog posts written for broad audiences, with undergraduate students specifically in mind. They also edit posts by other writers. ScienceBites is working to form a centralized online hub to aggregate work from early-career scientists writing at many different Bites Sites, and to provide support and advice to students looking to start new Bites Sites in additional disciplines. When our website is launched, we also plan to disseminate free online resources that will help to train new Bites writers and other science writers to communicate effectively with non-experts. Here, we will introduce SETAC attendees to our growing network of science blogs, which currently features 14 websites in 3 languages. We will also summarize what we have learned thus far about creating engaging science blogs, outline our future plans to grow ScienceBites, and provide information for any graduate students interested in joining an existing site, or starting a new one!

**513 Indirect Effects of Fishing Regulations on Human Mercury Exposure Pathways**

*J. Collins, A. Miano, AECOM; R.G. Stahl, E.I. DuPont de Nemours and Company / Corp Remediation; M. Liberati, E. I. du Pont de Nemours and Company*

Fish consumption advisories have been in place for more than four decades for a 24-mile reach of the South River, beginning at the former DuPont Waynesboro Virginia facility due to legacy mercury. There is currently a consumption ban in effect for all fish species with the exception of stocked trout, which are routinely monitored for total mercury (THg) concentrations by the Virginia Department of Environmental Quality and are considered safe to eat. The Virginia Department of Game and Inland Fisheries recently modified the trout fishing regulations for a portion of the South River in the City of Waynesboro to a Catch and Release (C&R) fishery to enhance the quality of the fishery. Previously, this reach was managed as a Delayed Harvest trout fishery, which permitted the harvesting of stocked trout from June 1st through September 30th. The change in fishing regulations to a C&R trout fishery may influence exposure durations of stocked trout, which may continue to accumulate mercury over time. A potential consequence of this change is that it creates the opportunity for anglers to illegally harvest and consume hold-over trout (i.e. stocked trout that survive through the summer months) in the C&R regulated area as well as legally consume hold-over trout that have migrated to other reaches of the river. To understand the potential impact of a longer exposure period of trout to bioavailable mercury in the South River, THg concentrations in hold-over, stocked trout were compared to the Virginia surface water quality criteria of 0.3 mg/kg THg in fish tissue. This presentation will provide a brief history of the legacy mercury contamination on the South River and the risk-management challenges associated with competing stakeholder goals. It will focus on how changes to fishing regulations may alter potential human exposure pathways, as well as the approach used to evaluate, document, and communicate potential changes to the site-specific risk/exposure paradigm.

**514 Rapid deployment of passive sampling wristbands in response to Hurricane Harvey: Community engagement and reporting**

*P. Hoffman, H. Dixon, Oregon State University / Environmental and Molecular Toxicology; D. Rohlman, Oregon State University / Environmental Health Sciences Center; L. Tidwell, K.A. Anderson, Oregon State University / Environmental and Molecular Toxicology*

Hurricane Harvey made initial landfall at San Jose Island TX, on August 25,2017. In some areas Harvey produced over 50 inches of rain, causing immediately flooding of low-lying terrain. On September 2nd several dams in the area released excess water, expanding the scope and duration of flooding. Thirteen Superfund sites were flooded and millions of pounds of hazardous chemicals were released. Multiple fires, explosions and chemical spills were reported. Researchers at Oregon State University (OSU) have been preparing for disaster research for several years, having in place an Institutional Review Board (IRB) approved 'Disaster IRB.' This IRB allowed OSU researchers to deploy chemical sampling technology quickly, with appropriate controls to ensure participants were safe and personal information was confidential. Immediately after safety in the area was ensured, contacts were established with collaborators in the Houston area. Equipped with the approved IRB and previously assembled 'go bags', developed for rapid field deployments, OSU personnel traveled to Texas. On September 20th, the OSU team conducted a training session with collaborators from multiple universities and enrolled volunteers from the highly-impacted Highlands, Baytown, Crosby and surrounding areas of Houston in a study in which participants wore passive-sampling silicone wristbands for one week during their Harvey clean-up process. Because of their small size, portability and ease-of-use, silicone wristbands are particularly suitable for post-disaster chemical sampling. Many participants were actively involved in clean-up during the sampling period. After one week, participants packaged and mailed their wristbands back to OSU. Even in the aftermath of Harvey, compliance was 85%, illustrating the approachability of this technology. Each wristband was analyzed for 1,529 chemicals, generating over 40,000 chemical data points for this initial study. In June of 2018, OSU personnel returned to Houston to communicate the results of the wristband analysis to participants. On average, participants had the highest number of chemical detections per wristband of any cohort examined to date. Additionally, OSU provided 200 wristbands to collaborators from Baylor School of Medicine and UTHealth School of Public Health for use in additional Harvey-related chemical exposure studies in which the initial disaster response chemical data will be paired with a follow-up non-disaster sampling campaign.

**515 Plastic bag bans in South Carolina: Science communication from grant proposal to informing public policy**

*A.D. Gray, University of North Carolina at Greensboro / Biology; J. Weinstein, The Citadel, Military College of South Carolina / Biology*

In 2014, the SC Sea Grant Consortium funded a proposal to investigate the sources, fate, and effects of plastic and microplastic pollution in Charleston Harbor, SC. As part of that proposal, the principal investigators (Drs. John Weinstein and Steve Klaine) proposed an outreach component that included communicating the results to stakeholders, the scientific community, and the general public. The results of this study were presented to local environmental groups (e.g. Audubon Society, Sierra Club), natural resource managers, and the scientific community. The principal investigators also worked closely with their respective on-campus Office of Communications/External Affairs to share the results with the general public through local media. Lessons learned from working with communication experts will be shared including the importance of using effective talking points and practice interviews. Communication strategies such as the timing of press releases, working closely with the media, and developing a "hook" for the story will also be shared. These outreach efforts, in part, led to several towns in coastal South Carolina banning single-use plastic bags and foam containers. Subsequently, these local bag bans resulted in the creation of House Bill 3529, also known as the Anti-Home Rule Act, which is currently being debated in the SC State Legislature. This act would ban local communities from passing bans on single-use plastics. While the primary intent of this funded research was to investigate plastic pollution and communicate our findings to stakeholders and the scientific community, through effective strategies, we were also able to communicate these findings to the general public. The impact of our outreach efforts far exceeded our initial expectations. This case study provides a compelling example of how effectively communicating science can influence public policy, and it underscores the value in making scientific results accessible to the public.

**516 Effective Science Communication – A Panel Discussion**

*J. Clarkson, Louisiana Department of Natural Resources; S. Sager, Arcadis U.S., Inc.; L. Paulik, Maul Foster & Alongi, Inc. / Environmental and Molecular Toxicology; A. Bonisoli Alquati, California State Polytechnic University, Pomona / Department of Biological Sciences; W.J. Berry, USEPA / Atlantic Ecology Division / ORD / NHEERL*

Examples of both successful and unsuccessful communication of scientific issues can provide opportunities to develop clear strategies for talking to stakeholders. Communicating science on topics such as risk assessment, risk management and policy development requires interaction between scientists and stakeholders. Government agencies, industries, and scientists have long employed structural approaches to communicate science, risk, and policy to interested parties. Risk assessment, adaptive management, structured decision-making and translational science may use different terminology and employ slightly different approaches, but they all stress the importance of meaningful communication with stakeholders before, during, and after projects. However, many scientists tasked with conveying the information remain uncomfortable in the role of science or risk communicators. The importance of effectively communicating with stakeholders is therefore often overlooked and risk communication remains one of the most underused tools for environmental management. This panel discussion will engage the audience and all the speakers for the session in a two-way conversation designed to highlight effective risk communication strategies as tools for use by science and risk communicators. The co-chairs for the session will moderate the discussion. Each presenter will provide a single summary slide to the moderators, who will review and collate the information. The combined summary will be used as a starting point for discussion and for drawing questions and comments from the audience. The moderators will also create a list of questions and comments for the panel, to ensure a robust and lively discussion is presented. If needed, the panel can respond to any questions that were not addressed during the individual presentations due to time constraints. Through our presentations and panel discussion, we will highlight ways to make science more accessible and useful to stakeholders. Ultimately this will promote more useful science, and greater public awareness of the importance of science.

## Micro- and Nanoplastic Methods for Environment Media

**517 Are Microplastic Measurements Comparable and Do They Reflect Reality?**

*W. Cowger, J. Guilinger, A. Gray, University of California Riverside / Environmental Science*

Accurately monitoring microplastic pollution in aquatic systems faces a number of challenges, from the technical details of sample collection and laboratory preparation, to the very metrics used to quantify abundance and character. Surface grab sampling is a common method of monitoring microplastics in rivers and streams. Utilizing previously published field data we investigated microplastic concentration profiles with hydrodynamic models. These models indicate that surface sampling can result in drastic over or under prediction of plastic abundance, depending on plastic type, size, and concentration. In the laboratory, density separation is a primary step for extraction of microplastics from various media, especially sediments and turbid waters. Average losses reported in spiked samples during density separation across laboratories range from 30-70%. We have found that even small changes in protocols can result in the difference between detection and non-detection in low concentration samples. Furthermore, count alone, the typical metric for measuring plastic concentration, does not provide the information needed for understanding the mass flux of microplastics and associated contaminants, and ultimately for effective plastic pollution management. We argue that morphometric information on microplastic particles is required for the estimation of useful metrics such as surface area, volume, and mass. Ultimately, standardization of monitoring, processing and measurement techniques will advance the comparability of results between laboratories, inform the assessment of environmental impacts, and develop effective management strategies.

**518 Recovery of microplastics from fine grain sediment with a common density separation technique using different agitation methods**

*S. Kell, College of Charleston / Grice Marine Laboratory; H. Mazur, The College of Charleston / Department of Geology; B. Beckingham, College of Charleston / Environmental Geosciences; J. Weinstein, The Citadel, Military College of South Carolina / Biology*

The most widely used method to extract microplastics (MPs) from sediment is a density separation technique described by Thompson et al. (2004) that uses a concentrated NaCl solution (1.2 g/cm$^3$) to float or suspend MPs so they can be decanted or filtered. This may be repeated multiple times to increase recovery, or used with other steps, such as chemical digestion by wet peroxide oxidation, to separate MPs from sediment. A majority of studies using a NaCl density separation have sampled coarse grain sediment, with recovery rates reported for spiked MPs. The objective of the present study was to test the effect of different mixing methods on the recovery rates of native MPs in fine grain sediment with high organic matter, which may bind MPs and impede separation. In addition, we focused on recovery rates of tire wear particles (TWP) which can be considered as an elastomer microplastic and have likely been under-reported in microplastic surveys in the U.S. We tested four mixing techniques (stirring, shaking, sonication and shaking/sonication) on 200 g of stormwater pond sediment and 2 L of solution in 4 L jars and repeated sequential extractions ten times. The supernatant was decanted through nested sieves (63, 150 and 500 mm) and treated to a mild hydrogen peroxide digestion (< 10% $H_2O_2$, 20°C). Microplastics were counted using optical microscopy. After the first extraction, more MPs were found in the order: shake > stir > shake/sonicate >> sonicate only. After four sequential extractions, method recoveries converged at 82.2 ± 1.8% (as % of total MPs after 10 extractions). Total microplastics extracted from the stormwater pond sediment sample (methods pooled, N=4) after 10 extractions was 12.0 ± 2.0 MP/g wet wt. On average, TWP comprised 75% of the total microplastics recovered; 95.8% were captured using shaking with four sequential extractions. We hypothesize that adherence to glassware, sweep flocculation during settling, and/or inability of the mixing methods tested to separate native MPs from mineral or organic agglomerations act to challenge recovery. In addition, we found tire wear particles are not compatible with wet peroxide oxidation conditions typically employed, and a milder treatment is required. This study highlights stormwater ponds as potential MP hotspots, as well as the need for testing and reporting of recovery rates of native MPs and further optimization of this low-cost and eco-friendly method for fine grained sediments.

**519 Magnetic Extraction of Microplastics from Environmental Samples**

*J. Grbic, University of Toronto / Ecology and Evolutionary Biology; B. Nguyen, University of Toronto / Department of Mechanical and Industrial Engineering; E. Guo, University of Toronto / Ecology and Evolutionary Biology; J.B. You, D. Sinton, University of Toronto / Department of Mechanical and Industrial Engineering; C.M. Rochman, University of Toronto*

Different methods to extract microplastics from environmental samples have been well researched, with methods like density separation, size separation, and chemical digestion emerging as the standard means of extraction. Still, small microplastics are often lost during the extraction process, and thus it is worth exploring complementary methods. We tested the ability of extracting microplastic particles from an environmental matrix by hydrophobicity. This was tested by surface modifying iron nanoparticles with a silane with a long hydrocarbon tail, and mixing them

into a sample where they bind to plastics via hydrophobic interaction. We then tested the efficacy of using a magnet to extract the plastics from the sample. We tested the method by spiking and recovering polyethylene and polystyrene in the size ranges of 10 – 20µm and had an average recovery of 92%. Additionally, we spiked and recovered polyethylene, polystyrene, polyurethane, polyvinyl chloride, and polypropylene in the size ranges of 300µm – 1mm and 1 – 5mm. The results for the 300µm – 1mm size range were an average recovery of 84% in reverse osmosis water and 78% in sediment from San Francisco Bay. For the 1– 5mm microplastics, the average recovery was 97% in reverse osmosis water. Overall, our novel method has relatively high recovery rates across different size ranges and media. Although further research can be done to work out some issues, such as the brute force of the magnet fragmenting microplastics, we believe the magnetic extraction procedure may provide a nice compliment to existing methods.

**520 Measurement of Microplastics using Pulsed Ultrasonic Extraction and Complementary Micro-spectroscopy Analyses**

*J. Wagner, S. Ghosal, Z. Wang, S. Wall, California Dept. of Public Health / Environmental Health Laboratory; M.B. Murphy, AAAS; H. Allen, W. Robberson, USEPA Region 9 / Superfund Emergency Response; A. Cook, USEPA Region 9; G. Robiou, E. Laird, USEPA / Office of Water; L. Stahl, USEPA / Office of Water / Office of Science and Technology*

Separation and characterization of microplastics < 1 mm in biological and environmental samples presents numerous analytical challenges. This work presents alternative sample extraction and analysis techniques used to identify microplastics as small as 15 um and address previously noted issues with other methods. Extraction methods that remove biomass matrices using aggressive chemicals can be problematic for the smallest microplastics, sometimes eroding their surfaces and creating reaction products that interfere with analyses. The current work instead utilizes ultrapure water and pulsed ultrasonic extraction to separate plastics from biomass without dissolving either, thereby preserving plastic surface characteristics, any adsorbed chemical species, and associated biota and ecological pathway information. This method was used to successfully separate microplastics from the stomachs of various species of freshwater and marine fish. Extracted particles were identified and characterized in terms of type, size, and morphology using complementary optical microscopy, scanning electron microscopy plus energy-dispersive x-ray spectroscopy (SEM/EDS), Fourier Transform infrared (FTIR) micro-spectroscopy, and Raman micro-spectroscopy (RMS). After optical and SEM/EDS screening, FTIR and RMS were used to identify specific plastic types. Shell pieces were identified in many fish stomachs that resembled microplastics, as were brittle, degraded plastics that were shattered like shells. Studies that rely on optical microscopy alone are thus prone to false positives and false negatives. Current progress and challenges for automation and optimization of these extraction and identification methods are discussed for various matrices, including method modifications for large freshwater fish, and separate analyses of prey fish inside larger fish stomachs.

**521 Method for Quantifying Microplastic Generation Rates from Various Plastics**

*J. Sipe, Duke University / Civil and Environmental Engineering; M. Wiesner, Duke University; N. Bossa, Duke University / Department of Civil and Environmental Engineering*

Microplastics have become an emerging new pollutant of rising concern due to exponential growth of plastic use in consumer products. Most microplastic pollution comes from the breakdown of larger scale plastics leading to the need of knowing how plastics behave in the environment. One way to study how microplastics are created in the environment is by using mechanical abrasion. A? custom abrasion machine has been built with novel features that provides data to calculate both generation rate and power?input. The generation rate of microplastics through abrasion across a variety of plastics ranging from biodegradable plastics ?to more persistent plastics in the environment (with and without nano?fillers) will be tested. These tests will determine the generation rate of ?microplastics from each plastic type using a constant power input and to ?identify plastic properties that drive their fragmentation. It is predicted that the plastics that fail faster under mechanical strength testing will produce more microplastics during abrasion and nano additives that add to the strength of the plastic material will produces less microplastics during abrasion. Currently, Polylactic acid (PLA), polyethylene glycol (PETG) and polycarbonate (PC) plastics have been tested using tensile tests and abrasion tests to calculate power input and abrasion rate. If 400 Joules of power is applied the abrasion rate is 110 g/m$^2$/min for PETG and 40 g/m$^2$/min for PC. This shows a correlation with PC being a stronger plastic than PETG. Results for the following plastics are also underway: thermoplastic rubbers, high impact polystyrene (HIPS) and polyvinyl chloride (PVC). The first overall power input and rate of generation to predict mass released of each type of plastic into the environment. Overall, this project will help comprehend and provide instrumental initial data to understand generation rates of microplastics from larger scale plastics and reduce microplastic pollution.

**522 Plastics in the salad? A first study for uptake and toxicity of nanoplastics in Lactuca sativa**

*A. de Souza Machado, Freie Universität Berlin / Institute of Biology; E. Faltin, J.B. Bachelier, Freie Universität Berlin; W. Kloas, Leibniz-Institute of Freshwater Ecology and Inland Fisheries; M.C. Rillig, Freie Universität Berlin*

Microplastic pollution is taking place in global ecosystems. However, there is a lack of toxicological information of the most reactive fraction of this novel stressor: the nanoplastics. This hampers risk assessments for environmental exposure, trophic transfer, and human health. We grew seedlings of *Lactuca sativa* (lettuce) for ~ 2 weeks in Hoagland hydroponic solution (control conditions) and then exposed individual plants to control conditions or to 6 different polystyrene nanoparticles encapsulated with red or blue fluorescent dye. The nanoplastic treatments were a factorial combination of 3 surface terminations (COOH, plain, and $NH_3$), 2 sizes (50 and 100 nm), and 2 concentrations (0.05 and 0.1 mg mL$^{-1}$). Plant evapotranspiration was monitored throughout the exposure. Seedlings (N=6) were harvested at 4 h, 8 h, 12 h, 24 h, 48 h, and 96 h of nanoplastic exposure, when the roots were removed from exposure media and washed in MiliQ water (10 min sonication, 35 kHz , 640 W, 10 mL). This root-wash and the hydroponic media were frozen. Plant parts (roots, shoots, or leaves) were separated, quickly freeze-dried and stored at – 80 °C. All plant tissues were later homogenized in phosphate buffer (pH 7.01, 0.1 mM). Fluorescence targeting the nanoplastic dyes was measured in the root-wash solution, hydroponic exposure media, and plant tissues. We quantified protein concentration and antioxidant capacity in plant tissues. All the nanoplastic types significantly affected plant physiology, with magnitude depending on particle properties and exposure concentration. The $NH_3$termination with 50 nm triggered the strongest effects on evapotranspiration and plant fresh weight. Generally, fluorescence decreased from exposure media to root-wash, root tissue, and then to other plant parts suggesting uptake that followed common temporal uptake patterns. However, this is not conclusive as the increase in fluorescence of shoots and leaves might be due to pigment changes of plastic-exposed plants. Also, confocal microscopy confirmed that most root fluorescence was due to nanoplastics strongly interacting with roots surfaces. Nevertheless, physiological changes in lettuce biomass, tissue protein, and antioxidant response were observed. Further research is needed to conclude whether this common dietary species would uptake plastics. However, the effects and their association with root fluorescence suggest that the idea of nanoplastics arriving at the salad plate cannot be discarded.

**523 Effects of waterborne and dietary exposures of nanoplastics in zebrafish: Maternal transfer, mitochondrial bioenergetics, and interaction with PAHs**

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Plastics are ubiquitous anthropogenic contaminants, including particles down to the nano-scale that are a growing concern in aquatic environments. The hazard of macroplastics is well documented, but micro and nanoscale particles remain relatively understudied. We have used zebrafish and polystyrene nanoparticles (PSNp) to understand the uptake and effects of nanoplastics on aquatic species. Initial experiments with embryos indicated that exposure to waterborne PSNp (0.1 – 10 ppm) caused early accumulation in the yolk sac, followed by the head, heart, and digestive organs. Despite such biodistribution, only minor signs of toxicity were detected, such as bradycardia and hypolocomotor activity. Further experiments also indicated that dietary exposure of adults to PSNp (as 10% of food weight) for one week did not affect their reproductive success, but PSNp were maternally transferred to the offspring. Both the adults and offspring had signs of impaired antioxidant system function, in addition to minor effects on the offspring embryonic development. Lastly, the interaction of PSNp on PAH toxicity was assessed in embryonic zebrafish. While embryos exposed to an environmental mixture of PAHs had signs of teratogenicity (pericardial edema and heart malformations) and impaired angiogenesis, once again waterborne PSNp exposure alone (0.1 – 10 ppm) did not cause significant effects on such parameters. Interestingly, PSNp co-exposure partially blocked these effects, which was correlated to reduced PAH uptake. The high surface area and hydrophobicity of nanoplastis likely facilitate PAH sorption, decreasing PAH bioavailability in our exposure condition. PAH sorption to PSNp was further investigated by spiking PSNp with a mixture of PAHs for one week, followed by exposure of zebrafish embryos to 1 ppm of spiked PSNp. PAH uptake and EROD induction were evident, but no signs of developmental toxicity were detected. In both experiments, PSNp seemed to be a biologically inert material, but they caused significant effects on mitochondrial function at larval stages: virgin PSNp (10 ppm) strongly decrease ATP linked mitochondrial respiration while PAH spiked PSNp (1 ppm) decreased mitochondrial spare capacity. Taken together, these data indicate that nanoplastics can: (i) accumulate via direct waterborne exposure, food chain and parental transfer; (ii) impair locomotor activity and mitochondrial bioenergetics; (iii) interact and modulate the toxicity of organic contaminants.

**524 An evaluation of the challenges and limitations associated with aquatic toxicity and bioaccumulation studies for manufactured particulate substances**

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The observations of physical effects in a variety of standardized and non-standardized ecotoxicological aquatic test systems in relation to exposure to nano-materials and microplastics challenges our interpretation of the potential risks associated with the use of such materials. There are a large number of stakeholders that have an interest in the results produced from toxicity tests. This results in a highly dynamic scenario which might limit the positioning and development of scientific test systems aimed at quantifying the differences between the intrinsic chemical toxicity mediated by molecular interaction between dissolved substances and biological receptors and physical effects associated with what is often mistermed 'particle-toxicity'. The example of microplastics is characteristic of a fast-moving issue that is likely to be regulated using hazard-based or precautionary approaches. The adoption of hazard-based regulatory instruments, however, should act as a warning, in that they impede the development of scientific tools aimed at assessing toxicity and risk for a wide range of both naturally and synthetically derived particulate materials. In this presentation, we demonstrate the importance of applying mechanistic understanding of the processes that influence exposure and effects of particles, differentiating between intrinsic chemical toxicity and physical effects. A key component in the development of mechanistic understanding is an appreciation of accurately defining the material under investigation, including the physical characteristics of size, shape, density, surface charge, as well as an understanding of its chemical nature. Based on an appreciation of the complex relationship between intrinsic and extrinsic properties that can occur within a test system, standardized approaches for creating and maintaining dispersions of particles and poorly soluble materials are urgently needed. In an effort to stimulate constructive dialogue between key stakeholders, a list of recommendations are reported for developing/interpreting aquatic toxicity tests with particles or poorly soluble substances that cause physical effects in test systems. These recommendations are meant for use beyond addressing nanomaterial or microplastic toxicity, but should be considered when encountering any material or chemical for which a physical effect is possible.

## Mechanistic Ecotoxicology: Bridging the Gap from Investigative to Regulatory Ecotoxicology

**525 Opportunities and challenges in the use of mechanistic ecotoxicology in ecological risk assessment**

*J.P. Staveley, E. Freeman, Exponent*

The measures of effect that are of concern in ecological risk assessment are those that relate to population-relevant endpoints (typically survival, growth and reproduction) because the goal is generally the protection of populations, not individuals. To characterize ecological effects of chemicals, data have historically been generated on individual species in laboratory exposures. We have expanded the field of ecotoxicology "up and out" in an attempt to obtain more environmental realism by, for example, looking at effects on multiple species in mesocosm studies. We have also expanded the field "down and in" to try to understand effects at sub-individual levels of biological organization and to find means to extrapolate this information to whole organisms and beyond. This is the essence of the Adverse Outcome Pathway (AOP) concept, which provides a useful construct for linking biological changes (key events) at the molecular, cellular, or tissue level, through processes at successively higher levels of biological organization to an observable adverse outcome, which is a biological change considered relevant for risk assessment. The development of High Throughput Screening (HTS) tests (in silico, in vitro, and small scale in vivo tests) offers promise in identification of key events. Similarly, information on toxicokinetics (TK) and toxicodynamics (TD) can explain the pattern of toxic effects over time by simulating the underlying processes, and can help expand our ability to characterize effects in individuals as well as at other levels of biological organization. Applications for these types of tools include chemical prioritization, development of QSARs, understanding differences in species sensitivities, read-across from one species to others, use in intelligent testing strategies, and assessing time-dependent toxicity or effects of fluctuating exposures. These tools thus hold the promise of reducing time, money, animal testing, and ultimately improving risk assessments. However, there are numerous challenges in building confidence that mechanistic ecotoxicology and HTS methods can ultimately be fully used in regulatory risk assessments to evaluate population-level impacts for ecological receptors. Distinguishing effects that may be adaptive or secondary effects from those that are adverse or primary effects, and translating these to outcomes for individuals and ultimately populations, will likely require a diverse set of tools and holistic thinking.

**526 Performance-Based Measures on Which EPA Relies in Determining the Utility New Approach Methodologies Using Defined Acceptance Criteria**

*E. Odenkirchen, USEPA HQ / Office of Pesticide Programs / Environmental Fate and Effects Division*

The Office of Pesticide Programs of the United States Environmental Protection Agency is committed to exploring new approach methodologies (NAMs) to prioritize testing, evaluate surrogacy, and establish effects endpoints for ecological risk assessment, provided the utilization of a NAM does not compromise the quality of the risk assessment process. This presentation outlines the background use, guiding principles and points to consider in establishing relevancy, reliability and confidence of NAMS for consideration in the ecological risk assessment process.

**527 Cross-species comparative analysis of Fish Short Term Reproduction Assay (FSTRA) data for the Endocrine Disruptor Screening Program (EDSP)**

*S.G. Lynn, USEPA / Endocrine Disruptor Screening Program; R.J. Bever, USEPA / Office of Science Coordination and Policy; K.L. Hamernik, A. Kamel, S.R. Matten, USEPA / Office of Science Coordination and Policy*

The US Environmental Protection Agency (EPA) established the EDSP to screen for chemicals that have the potential for adverse effects on the estrogen, androgen or thyroid systems. EDSP utilizes an 11 assay Tier 1 screening battery, which includes the FSTRA. The FSTRA is a 21-day aquatic exposure assay designed to measure fish reproductive success and other, potentially mechanistic, endpoints. The EPA guideline (OPPTS 890.1350) specifies the use of fathead minnows (FHM) while the OECD guideline (TG 229) also allows the use of Japanese medaka (JM) or zebrafish (ZF). The EPA is investigating cross-species sensitivity and variability of chemical toxicity testing data to facilitate assessment of endocrine bioactivity across wildlife species. FSTRA studies were conducted using all three species and survival, fecundity, plasma vitellogenin (VTG) concentrations (FHM & ZF), and liver mRNA (*vtg*) expression (JM & ZF) were measured along with other endpoints. Four (4) chemicals were tested: bisphenol A (BPA), 2-ethylhexyl-4-hydroxybenzoate (2-EHHB), triclosan (TCS), and tetrabromobisphenol A (TBBPA). These are well studied chemicals with ToxCast estrogen receptor (ER) bioactivity data available. These studies were conducted across multiple labs and inter-lab variability for control fecundity and body weight will be presented. Preliminary analyses indicate only TCS and BPA had significant effects on survival at the highest concentrations tested (based on measured concentrations) for JM (179 µg/L) and FHM (1300 µg/L), respectively. LOECs (µg/L) for fecundity are: BPA – JM (< 7.29), FHM (460), ZF (830); 2-EHHB – JM (< 2.98), ZF (42.2), FHM (>110); TCS – ZF (1.3), FHM (14), JM (57.5); and TBBPA – ZF (>276), FHM (366), JM (>1970). BPA and TCS show a greater than 10X difference in fecundity LOECs between the most sensitive and least sensitive species, which vary by chemical. Both BPA and 2-EHHB produced responses in VTG and/or *vtg* in all three species. BPA produced a response in male liver *vtg* at a lower concentration than fecundity only in ZF (< 21 µg/L). While 2-EHHB produced a response in male plasma VTG at a lower concentration than fecundity only in FHM (53 µg/L). BPA and 2-EHHB have reported ToxCast ER bioactivity scores of 0.45 and 0.37, respectively. Results support an estrogenic mode-of-action for BPA and 2-EHHB, but also demonstrate species-specific differential sensitivities across the four chemicals. *This abstract does not necessarily represent USEPA policy.*

**528 Response-response versus dose-response: Research to enhance the utility of adverse outcome pathways for regulatory decision-making**

*D.L. Villeneuve, USEPA / National Health and Environmental Effects Research Laboratory*

The adverse outcome pathway (AOP) framework was designed to aid the interpretation and extrapolation of mechanistic ecotoxicology data to outcomes of regulatory significance, typically measured at higher levels of biological organization. To date, AOPs have been used to support screening and prioritization of chemicals for further testing, guide more hypothesis-driven and tiered approaches to testing, inform the development of alternative assays, and help organize and interpret diverse toxicological data sets. However, many regulatory applications require the ability to generate quantitative estimates, bounded by some measure of uncertainty, of the probability or severity of an adverse outcome. Ideally AOPs could be used to help derive those quantitative estimates of apical effect based on non-traditional or mechanistic data obtained from so called "new approach methodologies" (NAM) or "alternative assays". However, most AOPs currently lack the kind of quantitative understanding required to facilitate quantitative estimation of biological effect thresholds. In part, this stems from toxicology's traditional focus on dose-response or concentration-response. Development of the models and understanding needed to support quantitative translation of NAM-derived data to estimated apical effects requires a shift in perspective in toxicological research and experimental design, away a sole focus on empirical characterization of dose-response, and toward the characterization of response-response relationships. This presentation will define and provide examples of response-response relationships and illustrate how they aid quantitative extrapolation along an AOP framework. Additionally, it will discuss the types of experimental designs that could be incorporated into mechanistic ecotoxicology research to better define response-response. Improved definition of response-response relationships is expected to enhance the utility of AOPs, and in turn expand the use of mechanistic data in regulatory decision-making. *The contents of this abstract neither constitute, nor necessarily reflect USEPA policy.*

**529 Role of in silico approaches in regulatory sciences: Mechanistic perspectives**

*V. Forbes, University of Minnesota / Ecology, Evolution & Behavior*

Much of the information used to assess the ecological risks of chemicals is gathered from standardized tests conducted under controlled laboratory conditions that measure individual organism responses in a handful of species. Growing pressures to reduce the use of vertebrates in toxicity testing, combined with methodological developments in high-throughput screening and in vitro technologies are encouraging new approaches to risk assessment. The Adverse Outcome Pathway (AOP) is being used as a conceptual framework to logically connect biochemical responses to chemicals, measured in vitro, to outcomes of relevance for risk assessment. Whereas an AOP framework can facilitate mechanistic insights into chemical impacts across different levels of biological organization, to be useful for estimating risk in a regulatory context, AOPs need to move beyond conceptual descriptors to quantitative, mechanistic models whose nonlinearities, feedbacks and uncertainties can be captured and communicated. In silico approaches, i.e., models, informed by relevant data, are needed to do this. Despite an extensive and growing body of literature documenting how mechanistic models can add value to ecological risk assessment, few have so far been used in a regulatory context. I will discuss reasons for this as well as some ongoing initiatives to address the concerns of the regulatory community and facilitate the implementation of in silio approaches in the future.

**530 Ecological models for pesticide risk assessment: How to balance complexity, uncertainty and practicality**

*P. Thorbek, BASF SE / Environmetal Safety*

Ecological risk assessments of pesticides are typically carried out by considering exposure estimated by e-fate modelling and effects estimated by laboratory experiments for standard species. However, with ecological models becoming more common risk refinement tools, several other factors are typically included in the risk assessment; e.g. life history, dispersal, population resilience, multiple stressors, landscape structure and temporal dynamics. This raises challenges for both registrants who have to identify which models can do this well and regulators who have to assess whether the models have been adequately designed, parameterised and applied. Using examples from bee modelling, model complexity, uncertainty analysis, good modelling practice and regulatory acceptance are discussed. The science of effect modelling is developing fast, but for routine use in regulatory risk assessments practicalities also need to be considered. Recommendations for model standardisation and development of baseline scenarios are given.

**531 A Chemical Prioritization Approach Based on EU Guidance for the Identification of Endocrine Disruptors**

*D. Feifarek, W.E. Hillwalker, A. Pastirik, SC Johnson / Global Safety Assessment & Regulatory Affairs; P. Mason, SC Johnson EurAFNE Limited / GSARA*

The European Food Safety Authority (EFSA) and European Chemicals Agency (ECHA), with support from the European Commission's Joint Research Centre (JRC), implemented guidance to identify endocrine disruptors in the context of biocidal products regulation (EU) No 528/2012 and plant protection products regulation (EC) No 1107/2009 in June, 2018. The guidance is entirely hazard-based and applies to active ingredients (*and* co-formulants in the case of biocides). It is, therefore, conceivable that chemicals may be pulled into the evaluation for which limited data are available. Three criteria are defined for classification of a chemical as an endocrine disruptor: adversity, endocrine activity, and a plausible link between the two. Here, we propose a system for evaluating the weight of evidence using existing data (e.g., adverse outcome pathways, in vitro mechanistic data) in the context of these criteria. The outcome is a ranking which may be utilized by the applicant to identify chemicals for which further evaluation is warranted.

**532 Life history traits and lipid profile changes after CP exposure**

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*Daphina magna* is a key freshwater species that, like many other animals, needs a balanced supply of nutrients to develop and reproduce successfully. However, not only food availability and quality but also exposure to certain environmental contaminants can affect the reproductive and developmental success of *Daphnia magna,* such as through the disruption of lipid homeostasis. Chlorinated paraffins constitute a group of complex industrial chemicals, divided into three categories based on their carbon chain length: short chain ($C_{10}$-$C_{13}$, SCCPs), medium chain ($C_{14}$-$C_{17}$, MCCPs) and long-chain ($C_{18}$-$C_{30}$, LCCPs). SCCPs have been added to the Persistent Organic Pollutants list by the Stockholm Convention, due to their persistence in the environment, bioaccumulation in wildlife and humans and toxicity towards aquatic organisms. Substitution of SCCPs by MCCPs and LCCPs is now underway. MCCPS and LCCPS are highly hydrophobic, with their log $K_{OW}$ ranging between 6 and 12, making their (necessary) aquatic toxicity assessment very complicated. Metabolomics approaches have shown that SCCPs are capable of disrupting the glycerophospholipid metabolism, fatty acid metabolism and purine metabolism in zebrafish embryos. Glycolysis, amino-acid metabolism β-oxidation disruption after SCCP exposure has also been described in HepG2 cells. To test the possible disruption of lipid homeostasis caused by CP-exposure in *D. magna*, we performed experiments following an adapted OECD guideline 211 (*Daphnia magna* reproduction test): first, through a stable exposure of our target chemicals through a passive dosing polymer, instead of a solvent carrier. Secondly, through the changing of the recommended algae-based diet established by the guideline (traditionally *Pseudokirchneriella subcapitata*) into a combined diet of *Eustigmatos magnus* and *Crytomonas sp. (*considered high quality food), in order to maximize the reproductive investment of the *D. magna* individuals. Neonates were exposed to two different CP technical mixtures (1 SCCP and another CP mixture covering all categories) at two different concentrations (50 and 100 μg/L), for 14 days. During this experiment, we recorded varied life history traits. After the experiment, the animals were collected for fatty acid profile analysis. This will allow us a better identification of the negative effects of chemical exposure on the reproductive output and lipid profile and, ultimately, aid the environmental hazard assessment of MCCPs.

## Modeling Oil and Chemical Transport in the Water Column After Spills

**533 Modeling oil in the water column after spills: An introduction to end-user needs and considerations for response and damage assessment applications**

*S.E. Allan, NOAA / Office of Response and Restoration*

After an oil spill, natural resource damage assessment efforts may rely on measured or modeled chemical concentrations in the water column to estimate exposure and injury to aquatic organisms. This data may also be important for emergency response, pre-spill planning, and risk assessments. Large-scale sampling programs that characterize three-dimensional chemical gradients over space and time may be logistically or financially infeasible. Models based on chemical and environmental parameters can fill gaps in field-collected data or allow spill observations to be translated into estimates of oil in the water column. A wide range of modeling tools are available for predicting oil concentrations in water under surface slicks and sheens. These models vary in their scale, resolution, data needs, outputs, and uncertainty. Understanding the range of tools that are available and their intended applications and limitations is critical for end-users. An introduction to needs and considerations for modeling oil concentrations in the shallow mixed layer of the water column will be presented in the context of their application to exposure and injury assessment and integration with other field-collected data and models. Examples from past spills will be presented and evaluated. This will provide context for an in-depth review and comparison of a range of newly developed and current use models.

**534 Water Column Exposure Modeling Tool**

*J.S. Bonner, Research, Applied Technology, Education, and Service, Inc.*

Considering the need for viable assessment modeling tools and the limited availability of empirical petroleum chemistry data from oil spills, we have developed a set of "simplified" modeling tools to estimate water column exposures of Total Petroleum Hydrocarbon (TPH) and 50 Polycyclic Aromatic Hydrocarbon compounds (PAH50) resulting from the entrainment, dissolution of surface oil into the upper water column and subsequent transport/and or ultimate fate of the relevant oil fractions. Oil from a surface slick is dispersed (or entrained) into the upper water column as a result of waves breaking on water surface areas covered with oil. The amount of oil entrained and dispersed is dependent on the oil thickness and viscosity, as well as sea state. The oil is injected to depths that scale with the wave height that is present during the entrainment period. Once oil is entrained at the surface, it is transported vertically through advection (stokes settling) and dispersion through the water column. Oil droplets in the near surface water column will rise back to the sea surface at rates dependent on their size and the oil buoyancy (i.e., Stoke's rise velocity). If turbulent mixing levels are sufficiently high, the oil droplets may remain in the water column, however, some portion of the oil entrained will rise to the sea surface during a model time step, with the cutoff droplet size (sizes larger than this value returned to the sea surface) dependent on the competing buoyant rise rate (allowing oil to resurface) and turbulent dispersion (causing the oil to remain dispersed subsurface). We have developed modeling tools to estimate surface oil entrainment/droplet formation/dissolution and upper water column concentrations of relevant petroleum hydrocarbons and attempted a simplified "inverse problem" to bracket the expected water column impact of these processes. We have also developed two additional tools to determine vertical distribution of fish eggs in the water column. These tools can be used together to determine petroleum hydrocarbons exposure of fish eggs in the water column. The vertical transport models for crude oil and/or fish eggs are based on the fundamental advection-diffusion equation.

**535 Modeling Near Surface Concentrations with a Lagrangian Element Oil Spill Model**

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The General NOAA Operational Modeling Environment (GNOME) is a particle tracking model developed and used by NOAA's Emergency Response Division for modeling oil spill fate and transport. GNOME was primarily designed to be used in emergency response and thus is very flexible in its use of inputs of the driving fields: winds, currents, and other parameters. Modeling in support of emergency response can be highly variable in scale and purpose: from predicting fate and transport for a small diesel spill to examining movement across the North Pacific of marine debris generated from the Japan tsunami. To support these broad use cases, GNOME has a model framework that allows multiple algorithms to be "plugged in" for different use cases, including behaviour of oils and other substances, 2 or 3 dimensional modeling, and various mixing algorithms, making it an excellent platform for exploring modeling approaches   Modeling the fate, transport, and dilution of petroleum products in the near surface presents a number of challenges. These include the fact that oil has many components with different solubilities, and thus forms surface slicks, droplets in the water column, and dissolved compounds, each of which are transported differently. In addition, the dynamics of the ocean surface are extremely complex, with effects from wind, waves, salinity gradients, heating and cooling, and other sub-mesoscale processes. Many of these processes are parameterized in oil spill models and hence may represent a significant source of variability among simulations.   Most oil spill fate and transport models use a Lagrangian element (particle tracking) approach. This paper will review the challenges of modeling oil concentrations in the near surface in general, and specifically the challenges of using a Lagrangian element approach. We will discuss multiple approaches for mixed layer modeling, and outline the methods used in the NOAA GNOME model. Examples from GNOME results will be used to illustrate the strengths and weaknesses of the approaches considered.

**536 Modeling Water Column Exposure Dynamics from Oil Spills: Issues, Model Approach and Validation**

*D.P. French-McCay, RPS Ocean Science*

For aquatic biota, quantification of oil and chemical exposure and effects is complicated by the high degree of spatial and temporal variability of in-water concentrations, as well as the organisms' distributions, movements, and sensitivity. Exposures to water column contamination above thresholds of concern are on the time scale of hours and spatial scales of meters. Further, oil is a mixture of thousands of hydrocarbons with varying physical-chemical properties and toxicity. As oil weathers in the environment, i.e., as evaporation, dissolution and (microbial and photo-) degradation ensue, the hydrocarbon composition in both the dissolved and particulate phases changes, as do the bulk properties of the oil, which affects fate and aquatic exposure. The SIMAP (Spill Impact Model Application Package) oil fate, exposure and effects model system has been developed over several decades to quantify oil and chemical exposures for support of risk assessments, emergency response decision-making, and natural resource damage assessment. The oil and chemical fate model tracks the movement of chemicals from oils and other mixtures using a Lagrangian approach, in which sublots and chemical components of the released pollutant mass are followed in space and time as they are transported, dispersed, and physically or chemically changed (i.e., entrainment, droplet/particulate formation, dissolution, volatilization, degradation, adsorption, etc.), accessing pathways of stressors and their concentrations at any desired/needed scale of spatial and temporal resolution. An integrated Lagrangian activity-based exposure model tracks the exposure history of individual biota to the mixture of chemicals through the affected environment using behavioral information and accounting for physical transport of plankton by currents. The response to the integrated exposure is evaluated with a pharmacokinetic-based toxicity model, accounting for the relative composition of the chemical mixture, as well as the influence of temperature and duration of exposure on the dose-response relationship. Example applications of this integrated model system for water column exposures after oil spills show their ephemeral nature, even for long-duration releases from a fixed source. Water column exposures estimated by SIMAP have been validated with field sample data from the North Cape and Deepwater Horizon oil spills.

**537 How mixed layer dynamics influences the evolution and exposure concentrations during an oil spill**

*C. Beegle, J. Skancke, T. Nordam, SINTEF Ocean / Environmental Technology*

The mixed layer is a dynamic feature of the ocean, which varies greatly by location and season. Even within the course of a day, the stratification in the upper water column can change with the differences in daytime solar heating and nighttime atmospheric cooling. Using the peer reviewed publications and available climatologies, we selected a variety of mixed layer profiles to explore the variety of conditions in the upper ocean around the world. Simple numerical oil droplet experiments, where droplets are released and allowed to rise in a given turbulence profile. We sample a variety of turbulence profiles so that we examine different mixed layer scenarios in order to show how these dynamics influence the amount and timing of oil surfacing. For example, as the mixed layer stratifies with either solar heating or freshwater input, vertical turbulence near the surface decreases, meaning that submerged droplets can have a higher probability of rising to the surface. These sample cases are designed to help provide intuition. More sophisticated cases are run in a fully 3D oil spill trajectory model. Using ocean circulation model output from various world regions and with different timing through the year, the variation is shown in how surface oil evolves under different conditions and in different areas. By using the 3D time dependent model, different oils, mixed layers and resulting exposures scenarios can be examined. Scenarios include a tropical, mid-latitude and Arctic case. A riverine case with freshwater over saline water is also examined. These 3D cases are run using the Oil Spill Contingency and Response (OSCAR) model, a highly peer reviewed oil spill trajectory, fate and effects model.

**538 Making sense of oil spill model results for the near surface water column: Extended capabilities of the Chemical/Oil Spill Impacts Model (COSIM)**

*S.M. Bartell, Cardno Entrix; J. Kubitz, Cardno; Y.B. Atalay, Cardno Entrix; J. Webber, J. Wakefield, Cardno; M.J. Fichera, ERM, Inc.*

Characterizing the ecological impacts of oil spills is a primary motivation for advancing capabilities in modeling oil spill transport and fate in the near surface water column. Increased accuracy in simulating spatial-temporal concentrations of oil chemical constituents are of minimal interest in absence of parallel developments in capabilities to transform these concentrations to estimated ecological effects. The Chemical/Oil Spill Impacts Model (COSIM) has advanced current oil spill risk assessment capabilities by including state-of-the-science algorithms to compute lethal and sublethal effects of oil constituents (hydrocarbon blocks) on organisms that inhabit the upper (~30 m) water column. An established target lipid model, empirical acute:chronic ratios, and an advanced phototoxicity algorithm combine to transform spatial-temporal concentrations of hydrocarbons (measured or modeled) to corresponding estimates of mortality and reduced growth for zooplankton, ichthyoplankton, and life stages of finfish defined by species life history and demography. A bioenergetics-based food web/ecosystem model appended to the core COSIM provides the capability to project the near-term (days) and longer-term (decadal) indirect ecological consequences of estimated direct lethal and sublethal population-level effects of oil exposure. The overall COSIM methodology addresses the highly variable distribution of biological productivity and marine resources in the upper water column and sensitive life history attributes (e.g., reproductive behavior) in assessing ecological risks and damages to resources. Basic life history information and population demographic data have been developed for more than 100 species commonly encountered in nearshore and offshore marine environments. This foundational database underpins the ability of the COSIM to assess the ecological impacts of oil spills. The COSIM will be presented as one methodology for adding assessment value to advances in oil spill fate and transport modeling in the near surface water column.

**539 Development of a Compound Particle Model for the Microbial Degradation of Oil Microdroplets Moving through a Water Column**

*G. Kapellos, Massachusetts Institute of Technology / Chemical Engineering; N. Kalogerakis, Technical University of Crete / Environmental Engineering; P.S. Doyle, Massachusetts Institute of Technology / Chemical Engineering*

After an oil spill in the sea, droplet clouds may be created either at the sea surface during the breakup of an oil slick by waves, or at the seafloor during the atomization of live crude oil extruding from a natural crack or a broken wellhead. Drifting droplet clouds pose a high risk of toxic effects to many marine species and, therefore, it is imperative to quantify the factors that determine their retention in the seawater column. We have recently developed a compound particle model for the biodegradation of solitary oil microdroplets moving through a water column (Kapellos et al., 2018). The compound particle is of the core-shell type and consists of an oily core that is successively surrounded by a bioreactive skin of negligible thickness and another bioreactive shell of finite thickness. The thin bioreactive skin represents a layer of superhydrophobic microbes that uptake oil directly from the oily phase, whereas the bioreactive shell represents a distinct biofilm phase. The new model accounts for all three modes of biodegradation: interfacial uptake, bioreaction in the bulk aqueous phase, and bioreaction in a biofilm formed around the droplet. Equations have been established for the determination of the droplet shrinking rate and the evolution of the compound particle dimensions as functions of the drifting speed, the microbial kinetics, the biofilm thickness, the diffusivity and solubility ratios. Numerical analysis is currently used to extend the domain of validity of the model by taking into account the effects of multiple oil components, oxygen limitation and biofilm erosion. Major findings of the theoretical analysis for the droplet retention in the water column will be discussed in conjunction with preliminary results from the biodegradation of hexadecane droplets by *Marinobacter sp.* in a microfluidic setting. Acknowledgements: This work has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 741799 (project "OILY MICROCOSM").

**540 Polar hydrocarbon compounds within surface water column following an oil spill**

*Y.B. Atalay, Cardno Entrix; J. Kubitz, Cardno*

Polarity is determined by how electrons within the atoms of a compound are arranged. In polar compounds, electrons are not uniformly distributed across the compound's structure and make certain parts of the compound more positively or negatively charged. Polarity underlies a number of physical properties including surface tension, solubility, and melting and boiling points. Some polar hydrocarbon compounds that contain nitrogen, sulphur and oxygen occur naturally in crude oils and refined petroleum products. In addition, weathering processes such as biodegradation and photo-oxidation have been shown to transform polycyclic aromatic hydrocarbons (PAHs) to oxygenated hydrocarbons (OxPAHs). In this paper, we will present a review of recent findings for polar hydrocarbon compounds and discuss how their environmental transport, fate and toxicity compare with nonpolar compounds, with specific emphasis on the near surface water column.

## Bivalves as Indicators of Exposure and Ecosystem Health

**541 Cellular Biomarker Responses of Freshwater Bivalves to Chlorpyrifos**

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Chlorpyrifos is the number one active ingredient used in many insecticides in the United States. The insecticide is mainly used for corn, soybean, and alfalfa agriculture, which are the top production crops in the US Midwest Region. The runoff from agriculture leads to very high concentrations of chlorpyrifos throughout the river basins of the Great Lakes, especially along the Maumee River basin. Environment concentrations of the insecticide often depend on proximity to the source, weather and when the insecticide was spray. Chlorpyrifos can have negative effects on freshwater ecosystems and organisms. Bivalves are valuable bioindicator organisms that can be used to characterize ecosystem health and monitor the effectiveness of remediation programs. However, many North American freshwater bivalves are threatened or endangered so studies with abundant invasive freshwater species such as *Corbicula fluminea* and *Dreissena polymorpha* were used to assess chlorpyrifos toxicity. One part of these investigations was in situ caging studies of *C. fluminea* and *D. polymorpha* conducted by the National Oceanic and Atmospheric Administration (NOAA) as part of the Mussel Watch program in the Maumee River Basin in Lake Eerie, in collaboration with the

Environmental Protection Agency (EPA). Cellular biomarkers (including acetylcholinesterase, lipid peroxidation and glutathione) of sublethal toxicity of clams and mussels from these field studies. Laboratory exposures of *C. fluminea* to environmentally relevant concentrations of 99% pure chlorpyrifos and commercially available Lorsban® were conducted at UNCC. Adverse effects in both bivalve species related to pesticide exposures, especially acetylcholinesterase, were observed. These studies will facilitate insights regarding the impacts and extent of chlorpyrifos on environmental health of freshwater ecosystems.

**542 Impact of environmental contaminants on aquatic life: Measurement of biomarkers of oxidatively induced DNA damage in dreissenid mussels by GC-MS/MS**

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Using gas chromatography-tandem mass spectrometry (GC-MS/MS) with isotope dilution, we identified and quantified in the DNA isolated from dreissenid mussels numerous oxidatively-modified DNA bases and 8,5´-cyclopurine-2´-deoxynucleosides. We found significantly higher concentrations of these potentially mutagenic and/or lethal lesions in the DNA of mussels from the polluted locations as compared to the animals collected at the reference site in the Great Lakes. These results align with the National Oceanic and Atmospheric Administration's (NOAA) data showing that elevated concentrations of toxic compounds such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), trace metals or miscellaneous substances were found in mussels within the polluted area as compared to mussels collected in the reference site. The measured DNA lesions can be used as biomarkers for identifying DNA damage in mussels from polluted and reference sites. Such biomarkers are needed to identify the bioeffects of contaminants in affected organisms, as well as to determine whether remedial actions have proven successful in reducing observed toxic effects. The NOAA, National Center for Coastal Ocean Science, Mussel Watch Program (MWP), uses dreissenid mussels to monitor chemical contamination in the Great Lakes. Some of the contaminants, such as pharmaceuticals, may cause oxidatively induced DNA damage at low/unmeasurable concentrations. Therefore, we may detect and quantitate DNA damage without being able to measure the contaminant if it does not bioaccumulate, making DNA damage important even if we cannot link it to measured contaminants. DNA damage would signal a need for forensic chemistry in other matrices (POCIS – Polar Organic Chemical Integrative Samplers or SPMD – Semipermeable Membrane Devices) that accumulate contaminants that do not bioaccumulate in mussels. In recognizing the need for both chemical and biological monitoring information, the NOAA's MWP partnered with the National Institute of Standards and Technology (NIST), to conduct a pilot project to implement alternative biomonitoring techniques that complement chemicals concentrations measurements in mussels.

**543 Monitoring Exposure and Impacts of Toxics in The Great Lakes Area of Concern Using Genomic Biomarkers of Dreissena polymorpha**

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Great Lakes Areas of Concern are contaminated with both legacy contaminants and emerging contaminants which may be the cause of environmental degredation. Determining the potential for biological exposure over time can be difficult. In addition, it is difficult to identify the compounds that may be the greatest cause of biological degredation can be difficult in a mixture of hundreds of compounds that may be present. This project assessed the relationship of chemical exposure to the expression of key molecular biomarkers indicative of stress to 1) determine their potential to predict exposure to specific chemicals in situ 2) establish the impact of exposure length on biomarker reliability 3) aid in the evaluating the health of the Niagara River compared to its tributaries. In addition global gene expression analysis was used to determine how entire pathways may be altered in relation to particular exposures. Caged zebra mussels were placed in seven tributaries of the Niagara River in conjunction with NOAA's NCCOS Mussel Watch Program. Mussels were collected at five and ten weeks to determine exposure to contaminants in AOCs including PCBs, PAHs and contaminants of emerging concern. Additional mussels were collected to evaluate the relationship of chemical exposures to mRNA biomarkers critical to detoxification, including P-gP, GST, AHR and HSP70. Contamination in the Niagara River was found to be less than in its tributaries. At five weeks of exposure in contaminated sites all the genes were down regulated from control. AHR and GST significantly correlated with PCBs and PAHs, and the CECs 4 nonylphenol and triclocarban over a gradient of contamination, suggesting that these biomarkers have potential for environmental monitoring. Mussels exposed for ten weeks were not correlated to chemical measurements, indicating that time influences the efficacy of these biomarkers. As triclocarban in particular seemed to be associated most strongly with gene expression patterns a second experiment evaluating the impacts of TCC on gene expression was conducted. Gene expression of AHR, GST and P-gP biomarkers indicates that field data is supported by single chemical exposure data and that TCC may be having a significant environment impact in these estuaries.

**544 Considerations for Metabolomic Evaluation of Dreissenid Mussels as an Indicator of Aquatic Environments in the Great Lakes**

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Environmental metabolomics focuses on detecting system-wide biochemical changes in organisms in response to environmental exposure. As ubiquitous, sessile, filter-feeders responsive to chemical contaminants, bivalves have been used as bioindicators in ecotoxicology. In order to validate this tool for risk assessment and environmental monitoring, it is important to ensure that the bioindicator will be vulnerable to exposure levels in the natural habitat; especially in ecosystems where chronic exposure levels may be low and physiological responses may be difficult to detect even with sensitive methods like metabolomics. In collaboration with the NOAA Mussel Watch Program, we evaluated dreissenid mussel metabolomes to assess aquatic environments in the Great Lakes. Whole-body polar extracts were characterized using nuclear magnetic resonance (NMR) spectroscopy and classified using multivariate statistics. We will present findings from two pilot studies at Milwaukee Estuary in Lake Michigan and Maumee River in Lake Erie. Specifically, we will discuss the challenges of validating an organism as an environmental indicator of exposure using a sensitive metabolomics approach. Spatial and temporal considerations, as well as handling effects on deployed organisms will be addressed. Additionally, metabolomic measurement methods for new matrices will be discussed in the context of technical reproducibility (sample preparation and instrumentation (specifically NMR)) and sample stability. Before experimental metabolic signatures of exposure can be compared, we must first understand the basic biology of the indicator organism at the location of interest and realize the variability and influence of biotic and abiotic factors. Acknowledging and comprehending these baseline data provides context for interpreting metabolic responses of exposure.

**545 Occurrence and spatial distribution of contaminants of emerging concern in Chesapeake Bay water, sediment, and oysters**

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The Chesapeake Bay is the nation's largest estuary, but few studies have evaluated the occurrence and distribution of contaminants of emerging concern (CECs) in this important body of water. The potential input of CECs from urban and agricultural sources has not been spatially evaluated. Furthermore, the accumulation of CECs into critical species, such as the Eastern oyster (*Crassostrea virginica*), has not been assessed. These knowledge gaps were addressed by monitoring a suite of 43 antibiotics, 11 hormones, 13 ultraviolet-filters (UV-filters), and a wastewater loading indicator (*i.e.*, sucralose) in water, sediment, and oysters from more than 50 sites in the Chesapeake Bay. Water samples were extracted using hydrophilic-lipophilic balanced solid-phase extraction (SPE) cartridges. Sediment and tissue samples were freeze-dried, extracted by modified-QuEChERS, and cleaned by reverse-SPE. All analytes were measured using liquid chromatography with tandem mass spectrometry. Results showed the ubiquitous presence of CECs in the Chesapeake Bay. For example, the norfloxacin (94.1 ng/L), enrofloxacin (17.8 ng/L), sulfamethoxazole (14.8 ng/L), and clarithromycin (9.7 ng/L) antibiotics were detected in the water phase. Estrone and four UV-filters, namely ethylhexylmethoxycinnamate, homosalate, octocrylene, and oxybenzone, were frequently detected in Chesapeake Bay water, sediment, and oyster tissue. High concentrations of estrone (58.4 ng/g) and 17β-estradiol (11.5 ng/g) were measured in sediment at the mouth of the Manokin River. Homosalate was present at concentrations as high as 187.9 ng/L in water, 74.2 ng/g in sediment, and 158.3 ng/g in oyster tissue. Sucralose was used to track the source of CECs in Bay areas. Spatial differences were contextualized using available land use data overlaid with the locations of wastewater treatment plants and animal feeding operations. These results demonstrate the ubiquitous presence of CECs in the largest estuary of the United States, and provide important motivation for studying the potentially deleterious effects of antibiotic resistance and endocrine disruption in the Chesapeake Bay.

**546 The environmental monitoring of operational discharges of oil and gas activity in the North Sea using caged mussels**

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Oil and gas companies operating on the Norwegian Continental Shelf are required to carry out environmental monitoring to obtain information on the actual environmental impacts of their activities and to give authorities a better basis for regulation. Scientists, operators and regulators have worked cooperatively for two decades in this program, for implementing knowledge, developing methods and tools to manage the offshore produced water (PW) discharges. A multidisciplinary approach (e.g. chemistry, biology, modelling and risk assessment) is in use at present to monitor discharges and reduce risks. In 2015, the Norwegian Environmental Agency published new guidelines as a result of the research activity performed since 1995. The new requirements have been applied for the first time in 2017. The holistic approach shows a significant improvement in the scientific outcomes of the monitoring, in a cost-effective way. The regulation includes mandatory monitoring using caged mussels and the use of biomarkers as early warning signals of potential adverse effects. A total of 19 stations were chosen: 17 around the PW discharged point, and 2 reference stations. The study design was based on the updated regional dose-related risk and effects management model (DREAM). At each station mussels were placed in mesh bags at a depth of 18 m. Mussels were collected after 6 weeks field exposure and the following parameters analysed: gene expression in gill, acetylcholinesterase, micronucleus assay, lysosomal membrane stability, histological evaluation of gills and gonads, survival in air and condition index. In addition, PAH and metal content were analysed as body burden. Summarising some of the results, the environmental monitoring study design was successful, with caged mussels exposed to the PW gradient following the forecasted plume. Higher level biomarkers, such as survival in air and condition index, did not show significant differences between mussels from the various stations. Lower level biomarkers (cellular), indicated a potential genotoxic effect of the PW discharge in organisms caged up to 100 m form the discharge point. The studied PW discharge point is in a highly active zone, where more platforms are present, the contribution of these platforms need to be considered.

**547 Measuring Cellular Energy Allocation in common bivalve species using Near Infra-red Spectroscopy**

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The function and sustainability of organisms, populations and ecosystems are driven by the input, use and transfer of energy. The storage and transfer of energy in organisms is influenced by environmental stress from physiochemical changes and contamination. Cellular Energy Allocation (CEA) is a measure of energy stores (protein, lipids and glycogen) versus energy consumption (ETS) and provides a measure of organism response to stress both natural and anthropogenic. The capacity to rapidly assess CEA would provide a valuable tool to assess organisms' responses to both physiochemical stressors such as temperature, salinity, pH and oxygen changes and anthropogenic influences. This study present an approach for rapid CEA measurements in five bivalve species (*Saccostrea glomerata* (Sydney Rock Oyster), *Ostrea angasi* (Flat Oyster), *Mytilus galloprovincialis* (Australian Blue Mussel), *Crassostra gigas* (Pacific Oyster and *Anadara trapezia* (Sydney Cockles) using near infrared spectroscopy

**548 Glutathione – Linkages Between Antioxidant Capacity and Sensitivity**

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Glutathione (GSH) is one of the most abundant antioxidants and also serves critical roles in numerous other pathways related to detoxification and scavenging of free radicals. The GSH responses of a variety of bivalves, both marine and freshwater, from a variety of locations around the world (southeastern US, Australia, Great Lakes USA, etc.) are presented. As a biomarker, both increased levels and depletion indicate pollutant exposure and stress. Numerous studies, including the zebra mussel and oyster studies presented here have shown increased GSH levels that are correlated to tissue levels of PCBs and PAHs. In contrast, depleted GSH levels have been observed in response to metal exposures and other stressors. Glutathione depletion can be especially problematic as it reflects that overall antioxidant capacity is significantly compromised, leading to greater sensitivity to additional stressors and widespread tissue damage. Similarly species or life history stages that have low GSH levels would be expected to be more sensitive to pollutant stressors. Our studies have indicated that freshwater bivalves have much lower baseline GSH levels than marine bivalves; the GSH levels of zebra mussels and Asian clams are an order of magnitude lower (approximately 1/10) than oysters and mussels. We have previously shown that larvae from GSH depleted parents are much more susceptible to pollutants. Glutathione serves as

a valuable screening level biomarker tightly linked to total antioxidant capacity that can be reliably measured and that provides valuable insights regarding pollutant effects and species-specific sensitivities.

## Novel Approaches for Understanding Diversity in Species Sensitivity to Chemicals

**549 Addressing species diversity in biotransformation: Variability in expressed transcripts of hepatic biotransformation enzymes among fishes**

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There is increasing evidence that diverse xenobiotic metabolizing enzymes exist among fishes, potentially resulting in different chemical sensitivities and accumulation, but this has never been systematically evaluated. One concern is that model test species such as rainbow trout, zebrafish and fathead minnows may not adequately represent the xenobiotic metabolizing capacity of other fish species. Our current study performed full-transcript, isoform sequencing on liver samples from two dozen phylogenetically diverse fish species. This novel RNAseq approach eliminated the need for transcriptome reconstruction resulting in reference genomes of the highest precision, allowing for detection of enzyme isoform orthologs among the species, as well as the nuclear receptors that control expression of the enzymes. Species were selected for broad phylogenetic coverage, as well as economic, research, and conservation importance, and included: sea lamprey (*Petromyzon marinus*), lake sturgeon (*Acipenser fluvenscens*), American eel *(Anguilla rostrate)*, alligator gar *(Atractosteus spatula)*, paddlefish *(Polyodon spathula)*, rainbow trout (*Oncorhynchus mykiss*), rainbow smelt *(Osmerus mordax)*, fathead minnow *(Pimephales promelas)*, Antarctic icefish (*Trematomus loennbergii*), common carp (*Cyprinus carpio*), and channel catfish (*Ictalurus punctatus*). Publicly available liver transcriptome data generated using the traditional Illumina RNAseq methods provided an additional 17 fish species, as well as mutual coverage for 5 fish species analyzed using the full-transcript read method. Together, the data suggest ostensibly important xenobiotic biotransforming enzymes, such as CYP1A orthologs are missing in some fish clades, while other enzyme families (e.g., CYP4V) are prevalently expressed across phylogenetically distinct fishes.

**550 Phylogenetic origins for differences in detoxification enzymes in fish and other vertebrates**

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Over the last decades, more and more examples have been discovered of perplexing differences in the expression of phase 2 detoxification enzymes in vertebrate species. These enzymes, also known as drug-metabolizing enzymes, comprise groups as the sulfotransferases, UDP-glucuronosyltransferases, and glutathione S-transferases. They are involved in conjugating a hydrophilic group to a poorly water soluble substrate, thus reducing the biological activity or toxicity of the substrate, and facilitating the excretion of the conjugated complex. To further investigate species differences in marine fish taxa, a variety of species was evaluated for the activity of sulfation and glucuronidation enzymes, using the natural hormone 17B-estradiol and the environmental toxicant 9-hydroxy-benzo[a]pyrene as substrates. Primitive fish species like hagfish and lamprey appeared to have no glucuronidation activity towards these substrates, while activity in sharks and rays was much lower than in bony fishes. This would indicate that the earliest vertebrates had no glucuronidation capacity, and that the array of glucuronosyltransferases that is known in more modern fish species and other vertebrates has evolved later. However, there are dramatic examples of other vertebrates that don't have glucuronidation capability towards phenolic substrates. We performed experiments to demonstrate that a number of snake species also lack phenol-type glucuronidation activity; while other reptilians like turtles and alligators had much lower activity than mammals. The lack of phenol-type glucuronidation enzymes, combined with the lack of N-acetyltransferase, leads to an accumulation of highly toxic aminophenol in snakes when exposed to acetaminophen, which phenomenon is used to control the invasive brown treesnake in Guam. In addition, even among the mammals it has been known that the felines lack phenol-type glucuronidation activity, which makes them extremely sensitive to compounds like acetaminophen. The explanation for this diverse expression of glucuronosyltransferases in different vertebrate taxa is probably a combination of phylogenetic origins and the degradation of genomic information in genes that are not essential, like phenol-type glucuronosyltransferases in obligate carnivores like cats, snakes and alligators.

**551 An non-targeted approach to evaluate endocrine disrupting activities of environmental contaminants in non-model species**

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Contaminants of emerging concerns (CECs) have been detected ubiquitously in aquatic environments, and their biological effects are problematic in the molecular and reproductive investigations at both field and laboratory. Especially, it is important to evaluate their long-term effects such as non-lethal endocrine disrupting effects in special concern species. However, it is very difficult to conduct whole organism exposure experiments in the threatened or endangered species. Therefore, it is critical to develop non-invasive assays customized for each species to evaluate endocrine activities on nuclear hormone receptors in vitro. To obtain sequence information, Hepatic or hepatopancreatic transcriptome of the lake sturgeon (*Acipenser fulvescens*) and freshwater mussels (Plain Pocketbook, *Lampsilis cardium* and Higgins Eye Pearlymussel, *Lampsilis higginsii*) were analyzed using the PacBio Iso-Seq method, which produces full-length transcripts *de novo*. Iso-Seq analyses obtained approximately 5000 of high-quality consensus sequences of each species. And then, Cogent analyses reconstructed approximately 2000 of the coding genome sequences from the consensus transcriptomes of these species. Blast2Go followed by InterProScan functionally annotated and classified these sequences. Identified 15 sequences of A. fulvescens nuclear hormone receptors were orthologous with androgen receptor, estrogen receptor, vitamin D3 receptor, peroxisome proliferator-activated receptor (PPAR), hepatocyte nuclear factor, chicken ovalbumin upstream promoter-transcription factor (COUP-TF) and orphan receptors. Four sequences of nuclear hormone receptors were identified each in L. cardium and L. higginsii. L. cardium sequences were homologous with hepatocyte nuclear factor 4, COUP-TF1, retinoid X receptor (RXR) α and γ, while L. higginsii sequences were homologous with ecdysone-induced protein 75b, COUP-TF1, PPARγ and RXRα. These nuclear hormone receptors need to be analyzed to assess the endocrine disrupting effects of CECs in liver and hepatopancreas. To avoid a potential artifact of different sequence affinity in the hormone response element among species, endocrine activities of CECs would be evaluated by quantifying transcriptional activities using a chimera protein of the ligand-binding domain and a universal DNA-binding. These assays would noninvasively reveal a potential long-term effects of CECs even in special concern species.

**552 A generalized individual-based model to assess sublethal impacts of contaminants on larval fish cohort growth and survival**

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There are thousands of contaminants and thousands of species to assess for contaminant exposure, making assessment of the impacts of these chemicals extremely complicated, expensive and time-consuming. Sublethal impacts of contaminants are often subtle and difficult to assess at higher levels of organization, and this is especially true for behavioral impacts. We developed a generalized individual-based model (IBM) that examines the sublethal effects of a contaminant, as measured through behavioral alterations, on a larval fish cohort growth and survival. On each simulated day, larvae forage for prey, grow, and are subject to starvation and predation mortality. Sublethal effects on behavior, as measured from laboratory studies, are incorporated into foraging via multipliers on larval swimming speed, capture success and handling time, and into predation mortality via the larval swimming speed multiplier. To understand which model parameters are most critical to model cohort survival and growth, we included a sensitivity analysis using a select suite of parameters that have previously shown to be sensitive in similar models. For this analysis, we explore the impacts of PCB126 and methylmercury (MeHg) on swimming speed in larval yellow perch (*Perca flavescens*). We tested the hypothesis that, while the sublethal impacts of contaminants on swimming speed are subtle, they can still have significant impacts on yellow perch cohort growth and survival. Further, we examined how well we could assess the sublethal impacts of these contaminants on larval perch in the face of model uncertainty. We hypothesized that the sublethal impacts on larval yellow perch swimming speed will be less than the uncertainty included in model. Future development of the model will include additional sublethal impacts on prey capture success and handling time. We will then expand this generalizable model to evaluate if model species like killifish (*Fundulus heteroclitus*) and fathead minnows (*Pimephales promelas*) respond in a similar manner as yellow perch to contaminants. This work will facilitate cross-species extrapolations and help determine risk to ecologically relevant populations.

**553 Cross-species Applicability of a Quantitative AOP Describing Inhibition of Aromatase Activity Leading to Reproductive Dysfunction in Fathead Minnow**

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Quantitative adverse outcome pathways (qAOPs) describe quantitative response-response relationships linking the molecular initiating event (MIE) and adverse outcome (AO). Such qAOPs enable quantitative prediction of the probability of occurrence or severity of an AO for a given magnitude of chemical interaction with an MIE. A qAOP has been developed for inhibition of cytochrome P450 aromatase (CYP19) leading to reproductive dysfunction through decreased circulating estradiol (E2) thereby reducing circulating vitellogenin (VTG). This qAOP was developed based on quantitative data from the fathead minnow (*Pimephales promelas*). However, different reproductive physiologies exist among fishes. Therefore, it was unknown whether a qAOP developed for fathead minnow would be predictive of reproductive dysfunction in other fishes. This study investigated whether this qAOP could accurately predict adverse responses to the model CYP19 inhibitor fadrozole, for three other fishes, namely Japanese medaka (*Oryzias latipes*), zebrafish (*Danio rerio*), and mosquitofish (*Gambusia affinis*). Japanese medaka and zebrafish have asynchronous oocyte development in common with fathead minnow, while mosquitofish have group-synchronous oocyte development. In vitro CYP19 inhibition assays demonstrate comparable sensitivities to fadrozole among fathead minnow, Japanese medaka, and zebrafish, while CYP19 of mosquitofish is 16-fold more sensitive. Results of 21-day reproductive assays demonstrate quantitatively comparable responses to fadrozole for E2, VTG, and egg production among the three asynchronous fishes. In contrast, fadrozole did not reduce E2 or VTG in mosquitofish and had limited effect on fecundity. Expression of CYP19 increased by up to 13-fold in the gonad of mosquitofish relative to up to 2-fold in fathead minnow. This suggests that mosquitofish, and potentially other group-synchronous fishes, have greater capacity to compensate for inhibition through up-regulation of CYP19. Overall, results of this study suggest that the qAOP developed for fathead minnow might be broadly applicable to fishes with asynchronous oocyte development, which includes numerous small-bodied fishes. But, the qAOP is unlikely to be applicable to fishes with group-synchronous oocyte development, such as catfish, trout, and sturgeons. This information could be essential in guiding more objective ecological risk assessments of fishes to aromatase inhibiting chemicals.

**554 Development of a neurobehavioral AOP for fish larva: Incorporating ecologically relevant behavioral alterations after exposure to PCB 126 and MeHg**

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Behavior is often measured in ecotoxicology studies because it is a sensitive endpoint that can indicate impairments after chemical exposure. However, it is challenging to link subtle behavioral changes to population relevant metrics and it is difficult to obtain this information on ecologically relevant species. We used the Adverse Outcome Pathway (AOP) as a framework to design laboratory and simulation studies that will tackle these issues. We conducted behavioral assays on four fish species to determine how differing sublethal levels of PCB 126 and methylmercury (MeHg) change larval fish behavior. Both traditional reference fish species such as zebrafish (*Danio rerio)* and fathead minnow (*Pimephales promelas)* were tested, as well as those more relevant to conservation such as yellow perch (*Perca flavescens)* and killifish (*Fundulus heteroclitus).* Methods focused on standardizing data collection and analyses to make them applicable to all fish species. Using video analysis, we measured behaviors on individual fish that can be translated to simulated population level outcomes (such as cohort survival and growth), and these behaviors include average swimming speed, overall activity and feeding rate. Preliminary behavior analyses of yellow perch indicate a non-linear response to increased chemical concentration for both MeHg and PCB 126 in total distance traveled, swimming bout frequency and duration, and that such responses are comparable between species after standardization. After behavioral assay analyses are complete, we will compare between all species and the relative impacts of contaminants on behavior will be used to estimate population level effects using individual based models (IBMs). Our approach demonstrates how the AOP framework can be used to develop measureable key events for neurobehavioral effects after exposure to contaminants that could predict population outcomes of ecological relevant species.

**555 Can genomic copy number variants inform us about within-species toxicant susceptibility variation?**

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Physiological variation induced by genomic copy number variants (CNV) have received tremendous attention in human disease research, but little work outside of this domain has been conducted. We used a model vertebrate system to establish the connection between genomic CNV and variation in toxicant susceptibility within a species. We assessed three zebrafish (*Danio rerio*) strains with variable toxicant susceptibility phenotypes using microarrays to identify CNV associated with PCB-126 induced gene expression differences (response eQTL) and confirmed our findings using targeted mutagenesis and a developmental toxicity assay. Using the 895 CNV found at high frequency within strains, the 124 differentially expressed mRNAs in females, and the 97 differentially expressed mRNAs in males (following 24 hours of 130 ng PCB-126/L), we identified two sex-specific response eQTL. The response eQTL in AB strain females modulates expression of *prpf4*, a gene associated with the splicosome and cellular stress, while the response eQTL in Tubingen (TU) strain males modulates expression of *dync2h1*, a gene associated with resistance to multiple compounds due to altered lipid partitioning. To test our hypothesis that CNV are drivers of PBC-susceptibility within species, we performed targeted mutagenesis using CRISPR-Cas9. Because the response eQTL in AB strain zebrafish appears to provide protection from the toxic effects of PCB-126 (AB are more resistant), we expected that CRISPR-mutant (crispant) AB zebrafish would show higher sensitivity to PCB-126. Conversely, the CNV in TU zebrafish associates with increased sensitivity, so we expected crispant TU to have higher resistance to PCB-126. Therefore we injected 1-cell stage embryos with CRISPR-Cas9 ribonucleic protein complexes targeted to the strain-specific response eQTL CNV, exposed embryos to 5-3125 ppb PCB-126 for 24 hours, and assessed developmental toxicity by heart rate, edema, and morphology until 5 days post fertilization. In support of our hypothesis, crispant AB zebrafish showed a reduction of $EC_{50}$ values by a factor of 10, from 627.7 to 67.9 ppb PCB-126, as compared to controls. Crispant TU zebrafish had slightly increased $EC_{50}$ values, from 35.3 to 47.0 ppb PCB-126, as compared to controls. This study shows clear evidence of CNV as drivers of the PCB-susceptibility variation within a species and is a first step towards inclusion of genomic CNV into modeling who or what will be susceptible to PCB exposure.

**556 Influence of life-history parameters on patterns of persistent organic pollutants in eastern North Pacific gray whales (*Eschrichtius robustus*)**

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The presence of persistent organic pollutants (POPs) in the marine environment is of global concern and have been associated with developmental dysfunction, reproductive failure, and immunosuppression in marine mammals. Research conducted in the 1980s-1990s reported POPs in eastern North Pacific (ENP) gray whale (Eschrichtius robustus) blubber but contemporary data were lacking. The current study examined blubber contaminant trends across various life history parameters including sex, age, and reproductive status in ENP gray whales. Blubber biopsies (n=121) were collected from free-swimming whales between 2003 and 2017 along the pacific coast of CAN, USA and Baja, California, MEX. To investigate maternal offloading, 19 mother and calf pairs plus 13 unpaired calves and 4 mothers were sampled. POPs and lipids were extracted from gray whale blubber samples using dichloromethane with accelerated solvent extraction, a previously validated analytical methodology. Sample cleanup was conducted using silica/alumina column chromatography and size-exclusion high-performance liquid chromatography. POPs including dichlorodiphenyltrichloroethanes (DDTs), chlordanes (CHLDs), hexachlorocyclohexanes (HCHs), polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) were determined in blubber extracts using gas chromatography/mass spectrometry. Blubber lipid content was determined gravimetrically. Mean POP blubber concentrations (wet weight) were $60 \pm 7$ ng/g ΣPCBs, $65 \pm 11$ ng/g ΣDDTs, $24 \pm 3$ ng/g ΣCHLDs, $18 \pm 2$ ng/g ΣHCHs, and $19 \pm 2$ ng/g ΣPBDEs. A subset of 96 samples and 18 analytes were used for statistical modeling and revealed significant life-history-related POP trends using linear regression of log-transformed congeners with multiple imputation and robust standard errors. Adult males had significantly higher POP concentrations than adult females, presumably due to maternal offloading. Young whales in this study had similar concentrations across sexes. Reproductively inactive adult females had slightly higher contaminant concentrations than females with calves. Mother and calf contaminant concentrations were similar although contaminant concentrations in calves were statistically higher. Our study provides current POP concentration data that enhances knowledge of POP trends across various life history stages in this protected species.

# Impacts of Unconventional Gas Operations on Surface Waters, Soils and Groundwater – An International Perspective

**557 Assessment of the likelihood of contaminant migration pathways in Australian coal seam gas basins**

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Coal seam gas (CSG) or coal bed methane production has grown exponentially in Australia over the last two decades with currently nearly 7,000 productive wells and another 18,750 wells planned over the next five to ten years. Concerns over environmental contamination by hydraulic fracture fluids (HFF) stem from (i) incidents involving poor surface handling of HFF and other fluids with potential migration pathways in soil and shallow groundwater, and (ii) leakage pathways arising from HFF injection into coal seams and unintended migration of these chemicals. A review of international studies on unconventional gas migration pathway studies was undertaken to provide prior knowledge for use in an Australian study. Whilst many of the leakage pathways considered in North American shale gas basins involved either (i) pre-existing fractures and faults, or (ii) up the production well due to poor casing or cementing, additional potential pathways highlighted for Australian CSG basins are, (iii) connectivity between CSG wells and water bores via a fracture in or extended out of the coal seam formation, and (iv) due to poor integrity water bores. Previous studies from the US have shown that natural pathways of fluid leakage exist between some shale reservoirs and shallow groundwater based on hydrochemical and environmental tracer data. However, analysis of micro-seismic and groundwater hydrochemistry data, and geologic modelling of vertical fracture growth reveal a low risk of leakage pathways developing in shallow aquifers confined by deep shale formations. This is due to limitations on the fracture growth vertically across aquitards often hundreds of meters thick, retention within the shale of limited amounts of injected fluid, and preferential fracture growth at shallow depths in the horizontal direction. The review identifies geologic criteria based on modelling and observations which support reduced risk of leakage pathways developing during CSG production in Australian basins. These include encountering certain favourable conditions during drilling/injection, i.e. (i) interfaces, such as natural fractures, faults, and bedding planes, which promote offsetting and branching, or rock layers with higher fracture toughness causing the cessation or reduction in fracture growth, (ii) narrow fractures due to a relatively higher elastic modulus, leading to higher viscous flow loss, (iii) high permeability layers, and (iv) overlying layer(s) with a higher confining stress.

**558 Release of geogenic contaminants during coal bed methane extraction: Laboratory studies**

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Concerns surrounding the use of hydraulic fracturing as part of the coal bed methane extraction process have mainly centred on the potential human health and environmental effects of the chemicals that are the constituents of hydraulic fracturing fluids. However, as part of their natural mineral composition, coal seams contain a number of geogenic contaminants that have the potential to be mobilised by the processes used to extract gas. These geogenic contaminants can be released from coal seams through the effects of chemicals such as chelating agents, acids, surfactants and solvents that are used in hydraulic fracturing. A laboratory-based study was conducted that investigated the potential for release of geogenic contaminants (inorganic and organic) from coal samples taken from locations across eastern Australia. Synthetic hydraulic fracturing fluid were prepared and used to leach coal samples under conditions representative of field conditions. The laboratory tests were designed to provide worst case estimates of the release of geogenic contaminants in the presence and absence of hydraulic fracturing chemicals. The logic for this approach was that if a contaminant was not detected during these laboratory simulations conducted under deliberately harsh conditions, then it was unlikely to be detected in samples obtained from an actual gas extraction operation. Detectable concentrations of over 42 trace elements were observed in the leach tests conducted. Based on comparisons with available surface water quality guideline values for aquatic ecosystem protection, the following inorganic geogenic contaminants were priorities for further investigations: Al, B, Cr, Co, Cu, Hg, Mn, Se, Tl, U and V. Phenol and cresols were the most commonly detected organic compounds particularly in the presence of the synthetic mixture of hydraulic fracturing chemicals. Higher concentrations of phenols and other low molecular weight total recoverable hydrocarbons in the $C_{10}$-$C_{14}$ range were released on leaching of coal samples with synthetic groundwater mixed with hydraulic fracturing chemicals compared to leaching with synthetic groundwater alone.

**559 Nontarget analysis of produced waters from two wells of different age in the Eagle Ford Shale Play, Texas, USA**

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Hydraulic fracturing is a major method for natural resource extraction that has become popular in recent years. The process works by pumping aqueous fluids into shales that are gas- and oil-rich. Fracking fluid is mostly made up of water and sand with the remaining fraction (less than 1%) containing chemicals such as surfactants, clay stabilizers and biocides. Flowback and produced waters (i.e. fracking fluid mixed with native released groundwater contain heavy metals and a variety of organic compounds) can potentially mix with nearby aquifers and/or surface water. Due to the lack of knowledge on aquatic contamination from this process, particularly for well of different ages of operation, a more advanced understanding of diverse constituents is needed. However, the proprietary nature and complex composition makes the analysis of fracking fluid even more challenging. A variety of analysis techniques will be needed to monitor the wide range of chemical constituents. Nontarget liquid chromatography high resolution mass spectrometry (LC-HRMS) is an emerging technique for analyzing complex chemical mixtures and has recently been used in various environmental studies. In our study, fracking flowback and produced water from two different wells from the Eagle Ford Shale play south of San Antonio, Texas, USA were selected for study. These wells varied in age by 2.5 years (well A had been in operation for 36 months, and well B in operation for 6 months). We employed standard toxicity identification evaluation procedures to fractionate samples from both wells and then analyzed these fractions using a nontarget LC-HRMS method. Preliminary results show that the methanol fraction contained a relatively large abundance of polyethylene glycol (PEG) species in both wells. A more in-depth analysis of the chromatograms showed that well A consisted of PEG species with longer retention times, while well B primarily consisted of PEG species with shorter retention times. Furthermore, Kendrick mass analysis (i.e. identification of homologous series) showed that well A and B consisted of 6 and 5 different PEG species, respectively. Thus far, the results from the nontarget LC-HRMS method have only identified PEG species. Nonetheless, the observed differences in the PEG species demonstrates that a nontarget LC-HRMS and Kendrick mass analysis method will be useful for providing PEG chemical fingerprint identifiers for fracking fluid and/or flowback and produced well water samples.

### 560 Chemical and bioassay assessment of waters related to hydraulic fracturing at a tight gas production site

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The aim of this research is to aid drinking water companies to evaluate the risks of hydraulic fracturing on drinking water sources with a focus on polar organic compounds due to their low removal efficiency during waste water treatment. Publicly available chemical assessments of hydraulic fracturing related waters are generally based on shale gas practices in the U.S., lacking information on other types of gas development also using hydraulic fracturing. This research fills this knowledge gap by presenting chemical and bioassay assessments of chemicals present in hydraulic fracturing related waters from a tight gas development in the Netherlands. Fracturing fluid, flowback water and groundwater from surrounding aquifers before and after the actual fracturing were analysed by means of HR LC-MS/MS, the Ames test and three CALUX bioassays (genotoxicity, oxidative stress response and polyaromatic hydrocarbon contamination). After sample enrichment a higher number of peaks can be found for both fracturing fluid and flowback samples. No clear differences in chemical composition were shown for the groundwater samples before and after hydraulic fracturing. Clear genotoxic and oxidative stress responses were found for the fracturing fluid and flowback samples. A preliminary suspect screening resulted in 25 and 36 matches in positive and negative ionization respectively with the 338 possible suspect candidates on the list. The results provide a scientific justification for the extensive measures currently in place related to the handling, transport and treatment of hydraulic fracturing related waters to avoid adverse environmental and human health impacts.

### 561 Ecotoxicity of Coal Seam Gas Hydraulic Fracturing Fluids

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With the increasing need for diversification of energy sources, the extraction of gas from coal seams has become a potential crossover energy provider in the move from coal to more sustainable energy sources for Australia and globally. As part of coal seam gas production, hydraulic fracturing of non or low producing wells is commonly undertaken. The toxicity of the individual major constituents that make up these fluids are generally well known, but the toxicity of hydraulic fracturing fluid as a mixture, or for many of the lesser/proprietary constituents is not well understood. The aim of this project was to understand the potential hazard of the hydraulic fracturing fluids in comparison with the in-situ formation waters to surface freshwater organisms in the gas tenements of the Surat and Bowen Basins of Central Queensland. The intent was to assess the ecotoxicity of the hydraulic stimulation fluids in the context of formation waters (pre-stimulation) and flowback water (produced water post-stimulation) from within the gas production areas. As a result, the program was designed in three phases. Phase 1 tested the formation water (water in the coal seam before stimulation) and source water (water used to formulate the hydraulic fracturing fluid) to establish background ecotoxicity. Phase 2 tested the incremental effect of hydraulic fracturing fluids mixed in laboratory water (of known ecotoxicity) and source water. Phase 3 tested the ecotoxicity of early and late stage flowback water from stimulated wells. The results of the project are presented along with the knowledge gained with regards to the hazard potential of the different fluid systems in use in Queensland at the time of the project.

### 562 Mahi mahi cardiomyocyte contractility and swim performance alterations following acute exposures to hydraulic fracturing flowback and produced water

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In North America, horizontal hydraulic fracturing (HHF) is an emerging industrial practice used to extract oil and natural gas reserves. However, little investigation has been performed on the wastewater by-product of HHF activities: flowback and produced water (FPW). Chemical characterization has revealed numerous potential cardio-respiratory toxicants to exist within FPW. The aim of this study was to determine if acute FPW exposures alter cardio-respiratory responses in organisms which may encounter FPW in a spill event, namely fish. Using the pelagic and extremely aerobic *Mahi mahi* as a model organism to characterize changes to organismal cardio-respiratory parameters, isolated primary cardiomyocyte contractile properties and swim performance were analyzed in fish acutely exposed to different fractions of an FPW sample collected from a well 2 hours post-stimulation. Overall, cardiomyocytes displayed expanded contractive events following exposure to 2% FPW dilutions, while no effects associated to salt and osmotic stresses were found. These expanded sarcomere contractive effects following FPW exposure were primarily the result of decreased myocytic relaxation velocities back to sarcomere baseline distances following initial contraction. This effect increased exposed overall cardiomyocyte 50, 75, and 90% sarcomere return to repolarized/baseline distance times. To follow up on cardiomyocyte effects observed, we plan to perform acute whole organism *Mahi* exposures, and determine if swim performance and swimming respiratory is impacted following FPW exposure. This is one of the first studies to mechanistically investigate FPW effects on cardio-respiratory parameters at the cellular level, and determine that even at relatively low dilutions, FPW may elicit effects on fish cardio-respiratory systems. Our results validate the cardio-respiratory system as an important endpoint to consider for FPW risk assessment and adverse outcome pathway analysis, and may be a system viable for exposure marker development.

### 563 Early Life Effects of Produced Water on Menidia Beryllina Juveniles

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The composition of produced water from extraction of gas and oil from shale is complex, containing the chemicals that were added to make it work effectively plus hydrocarbons and salts that vary depending on the geochemistry of the site. Dissolved solids can range from 100 mg/L to 400,000 mg/L. We tested produced water from two wells in Texas that were from the same geological formation but differed in length of time of operation. One was in operation for 6 months and the other for 3 years. Raw waters were characterized for various chemical parameters and fractionated by solid phase extraction following usual EPA procedures for toxicity identification evaluations. Ten-day old Menidia beryllina juveniles were used in 72 h acute toxicity tests of both raw samples at 10%, 3%, 1%, and 0.3 % and SPE fractions at 100%, 10%, and 3% initial concentrations, respectively. Exposures were conducted using 8 juveniles/dish and 6 replicates per treatment. Fifty percent of the exposure solution was changed daily. The MEOH extracts of the fractionated water samples were the most toxic in terms of fish survival. Fish exposed to sublethal concentrations of the raw waters were evaluated for gene expression alterations using RNAseq. Cyp1A, StAR-related lipid transfer protein and long chain fatty acid transporters were among the genes most highly up regulated by the exposures while DNA repair protein RAD 51 was the

most down-regulated transcript. Gene set enrichment analysis suggests pathways related to cancer, cardiomyopathy, ROS generation and inflammation may be sublethal targets of the exposures.

**564 The effect of hydraulic flowback and produced water salinity on juvenile rainbow trout (Oncorhynchus mykiss)**

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The extraction of hydrocarbons trapped in deep sedimentary deposits by hydraulic fracturing (HF) results in the formation of a wastewater named Flowback and Produced Water (FPW). This effluent is enriched in salts, organics and metals, and as such, is potentially toxic to aquatic biota. To date, the chronic effects of FPW exposure on fish have not been studied. Using an important model species, the rainbow trout, *Oncorhynchus mykiss*, the effects of exposure to either 3% FPW or its parallel saltwater-matched control solution (SW; i.e. salt component with no organic or metal toxicants present) were investigated. We measured the levels of plasma ions ($Na^+$, $K^+$, $Cl^-$, $Ca^{2+}$, $Mg^{2+}$), the activities of two key osmoregulatory enzymes, $Na^+$-$K^+$ ATPase and H-type ATPase. These biochemical endpoints were paired with gill histological analysis. Our results showed significant changes in gill morphology following exposure to FPW. These included the appearance of lamellar clubbing and an increase in the interlamellar cell mass. Although previous studies suggest changes in gill structure are driven by salts, no effects were observed in the SW group, suggesting an effect driven by either or both of the inorganic or organic toxicants. Furthermore, all effects on gill structure were only observed in 48-h exposed fish, with an apparent reversal of histopathology noted at later time points suggesting an ability of the fish to acclimate to FPW exposure. This study provides important insights for risk assessment, biomonitoring of FPW spills and minimization of post-spill environmental effects.

## Environmental Mercury Exposure: Mechanisms Associated with Its Immediate and Generational Effects

**565 Impacts of methylmercury on the physiology and behavior of a marine forage fish**

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Methylmercury (MeHg) is a neurotoxin that is found in fish tissues and bioaccumulates in aquatic food webs over a wide range of geographic areas. The extent to which fish exposed to food with high MeHg concentrations are impacted is largely unknown, particularly in marine fish. Previous studies on freshwater fish have suggested that MeHg concentrations can cause adverse effects on growth, behavior, reproduction, and other sublethal endpoints. Some species of marine fish have high tissue concentrations of MeHg but effects on behavior and reproduction are little known. Here we examined effects of MeHg to a marine fish at the larval stage, the sheepshead minnow *Cyprinodon variegatus*. Fish were exposed to MeHg through diet or maternal transfer. For dietary uptake, larvae (ages 7 d to 5 weeks) were fed commercial fish flakes containing 0 or 4.8 ppm MeHg. Growth rates, respiration rates, and swimming activity were tested. For evaluating maternal transfer of MeHg, the female juvenile fish were fed control and MeHg-contaminated diets from an age of 3 -5 months. After exposing the fish to MeHg, female fish were paired with Hg-free male fish for spawning. We assessed egg production, the developmental success of embryos, hatching success of embryos, survival of larvae, growth of larvae and swimming behavior of larvae. Data to date suggest that dietary MeHg has no significant impact on larval fish growth and swimming activity (swimming speed, acceleration, active time and swimming distance) even when their tissue MeHg concentrations greatly exceed 0.3 ppm, the reported threshold for toxicity in freshwater fish. MeHg had a small but significant impact on the respiration rates of these fish. Decreased egg production was not observed after dosing juveniles. Future data gathered on developmental and hatching success of embryos, survival, and swimming of larvae will allow us to further evaluate the transgenerational effects of MeHg.

**566 Mercury bioaccumulation and body size influence peripheral blood leukograms in the Brown Watersnake (*Nerodia taxispilota*)**

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Mercury (Hg) is a toxic heavy metal capable of polluting aquatic environments. In vertebrates, Hg can bioaccumulate, biomagnify, and maternally transfer to offspring. Exposure to Hg is known to negatively influence immune status, growth rates, reproductive success, behavior, and survival in numerous species. Risk assessments have investigated Hg bioaccumulation and effects in a variety of taxa, but little is known about its impact on reptiles. Brown Watersnakes (*Nerodia taxispilota*) exhibit traits that may put them at risk for accumulating significant amounts of Hg. They are long-lived (> 10 years), have a piscivorous diet, and are major predators in streams and rivers of the southeastern United States. This present study investigated total Hg (THg) levels in tail tips, the relationship between snout-vent length (SVL) and tail THg, and the impact of THg on peripheral blood leukocytes in *N. taxispilota* captured on the Savannah River. Snakes sampled in this study were downstream of a known Hg release source. Similar to other vertebrates, there was a significant increase in tail Hg levels with increasing snake size (ANCOVA, SVL: $F_{1,36} = 46.71$, $p < 0.001$). Although health criterion for THg in snakes is not available, mean THg levels in over half of snakes we sampled exceeded the USEPA's human health criterion for methylmercury in fish. Tail THg levels were inversely correlated with total white blood cell numbers ($r = -0.29$, $p = 0.078$). Further, as snake size increased, absolute basophil ($r = -0.43$, $p = 0.007$) and lymphocyte numbers ($r = -0.37$, $p = 0.023$) significantly decreased. These preliminary results from our first field season (2017) suggest a link between increasing THg levels in aging *N. taxispilota* with declining select leukocyte numbers, implying that these snakes, as they mature, may be more susceptible to infections as a result of continuous environmental and dietary Hg exposure. We will present our findings for both the 2017-2018 field seasons, adding blood THg and Se concentrations – allowing us to explore the relationship between Hg and Se concentrations in *N. taxispilota*.

**567 Systematic Review of Mercury Exposures Worldwide – Findings from the 2018 UN Global Mercury Assessment**

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The Minamata Convention on Mercury Pollution provides a mandate to take action against global mercury pollution. However, our knowledge of mercury exposures around the world remains limited. As part of the 2018 UN Global Mercury Assessment, WHO led the current study to increase worldwide understanding of human exposures to mercury by collating and analysing mercuryconcentrations in biomarker samples via a systemic review. A systematic search of the peer-reviewed literature was performed with several a priori search strategies set. The overall work captured 424,884 mercury biomarker measures taken from 336,015 individuals represented in 312 articles from 67 countries. We identified four populations of concern for which there exist a relatively robust dataset: 1) Arctic populations (mainly Inuit) who consume fish and marine mammals; 2) tropical riverine communities (especially Amazonian) who consume fish, and in some cases may be exposed to mining operations; 3) coastal and/or small-island communities who are avid seafood consumers; and 4) individuals who either work or reside amongst artisanal and small-scale gold mining (ASGM) sites. Individuals in select background populations worldwide with insignificant exposures to mercury sources have blood mercury levels that generally fall under 5 mg/L and urine mercury levels that fall under 3 mg/L. This systematic review documents that all people are exposed to some amount of mercury, and that there is great variability in exposures around the world. This type of information is critical in helping understand exposures particularly in light of the Minamata Convention on Mercury Pollution and certain stipulations within the Convention text (e.g., can inform Article 22's effectiveness evaluation especially for vulnerable populations; gauge changes over geographic space and time).

**568 Selenium Availability in Freshwater Fish is Inversely Related to Methylmercury Bioaccumulation and Toxicity**

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Based on the biochemical mechanisms of mercury (Hg) toxicity, concomitant assessments of selenium (Se) intakes are required for reliable assessments of risks potentially associated with Hg exposures. This consideration is especially true in regions that are Se-poor or exposed to high Hg inputs. In particular, subsistence consumers of high methyl-Hg ($CH_4Hg^+$), low-Se freshwater fish may be at greater risk than would have been assumed based on earlier assumptions that did not consider Hg's biochemical mechanisms of toxicity. Because $CH_4Hg^+$ is uniquely able to: 1) diminish maternal Se redistribution across the placental barrier, 2) decrease Se-transport across the blood-brain barrier, and 3) irreversibly inhibit selenoenzyme activities which are required to prevent and reverse oxidative damage in brain tissues, Se status is a pivotal consideration in $CH_4Hg^+$ risk assessments. The Hg and Se content data of ~5,000 freshwater fish from North American watersheds were compiled for calculation of their Health Benefit Values (HBVs). There was a strong inverse relationship between environmental Se availability and $CH_4Hg^+$ contents of the fish, especially among aquatic apex predators. Due to the high binding affinities between Hg and Se, the same chemical reactions responsible for the irreversible inhibition of Se-dependent enzymes, formation of insoluble HgSe, and the physiological consequences of high $CH_4Hg^+$ exposures were predicted to result in an inverse relationship between Hg and environmental Se availability. In the presence of adequate Se, Hg that is retired as HgSe cannot bioaccumulate in aquatic food webs. However, in the absence of adequate Se, there is no Hg retirement, and increased $CH_4Hg^+$ bioaccumulation should occur. This study found the highest fish Hg concentrations were observed in watersheds with the poorest Se availability. Although increased exposure from eating such fish will increase risk, the fact that Se-poor consumers will be more vulnerable to the effects of high $CH_4Hg^+$ exposures accentuate the risks of adverse effects. Thus, increased $CH_4Hg^+$ exposures in areas where environmental Se is poorly available may result in an adverse synergy with the potential to cause far greater impairments to fetal neurodevelopment than those predicted by earlier $CH_4Hg^+$ risk assessment criteria. Increased monitoring is required to identify regions that are likely to have subsistence populations at risk.

**569 Wildlife as a Surrogate Indicator for Impacts of Mercury on Ecosystem Health**

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Methylmercury (MeHg) is a cause for concern for the health of humans, wildlife, and the ecosystem due to its known immunotoxic, nephrotoxic and neurotoxic effects. MeHg bioaccumulates and biomagnifies in the aquatic food web putting the organisms higher in trophic position at greater risk of mercury exposure. The relationships between mercury (Hg), MeHg, inorganic Hg (iHg), and selenium (Se) exposure, and neurochemical and molecular biomarkers in beluga whales (Delphinapterus leucas (Pallas, 1776)) were characterized. Samples were collected in 2008 (n = 20) and 2010 (n = 15) in the Canadian Arctic from hunter-harvested beluga whales. Total Hg concentrations in the temporal cortex and cerebellum were 21.0 ± 22.6 mg kg−1 dry weight (dw) and 14.9 ± 18.9 mg kg−1 dw, respectively. Monoamine oxidase (MAO) activity was predicted by MeHg and the molar ratio of total Hg to Se (Hg:Se, $p < 0.05$); MAO-A mRNA transcription levels were predicted by iHg and Se ($p < 0.05$). Muscarinic AChR binding was predicted by MeHg ($p < 0.05$) and Hg:Se molar ratio ($p < 0.05$), and mRNA transcription levels of mAChR m1 was predicted by the Hg:Se molar ratio ($p < 0.05$). These results suggest that the cholinergic and dopaminergic signaling pathway in Eastern Beaufort Sea beluga whales may be sensitive to MeHg exposure. The response of beluga whales to MeHg exposure at a physiological and population level remains to be elucidated.

**570 Improving the Utility of Tetragnathid Spiders as a Bioindicator: Implications for Mercury, Methyl-Mercury, and Stable Isotopes**

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Tetragnathid spiders (Family: Tetragnathidae, Genus: *Tetragnatha*) found in riparian habitats feed primarily on emergent aquatic insects and have recently been used as bioindicators of sediment contamination and insect-mediated contaminant flux. To better understand the utility of tetragnathids as bioindicators, we designed a two-tier study in Tennessee's Appalachian Mountains. We first investigated the inter-site differences of whole-body tetragnathid stable carbon ($\delta^{13}C$*) and nitrogen (*$\delta^{15}N$*) isotopes, as well as*total mercury and methyl-mercury concentrations. We then investigated the utility of different tissues (legs, opisthosoma, and prosoma) to represent whole-body $\delta^{13}C$ *and* $\delta^{15}N$*,*and whether sex effected total mercury concentrations. Analysis of δ13C and δ15N revealed site-specific isotopic signatures, but tissue samples (legs, *prosoma* and *opisthosoma*) were not significantly different, and all tissues were representative of whole-body composites. Results showed that total mercury concentrations were different among sites, but not between sexes. There was no difference in the MeHg concentrations; however, the percentage of total mercury that was methyl-mercury ranged from 17-47 %-MeHg – well below previously reported values of ~70%-MeHg.

**571 Using *Caenorhabditis elegans* to explore novel pathways involved in methylmercury's effects on cellular energetics and neurodegeneration**

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Methylmercury (MeHg) is a major environmental contaminant that has been linked to developmental and neurological disorders. With the aging population, new concerns have arisen on how MeHg may affect aging and age-related diseases such as Parkinson's disease (PD). Studies of MeHg toxicity have traditionally been performed in mammalian systems, which are both costly and complex for age-related studies. The soil-dwelling nematode, *Caenorhabditis elegans*is a valuable alternative to both rodents and mammalian cell culture for studying the effects of MeHg on age-related conditions. *C. elegans* has a short lifespan, well characterized genetics, highly genetically manipulable, and highly homologous to humans. Taking advantage of the system, our lab has shown that MeHg behaves similarly in nematodes as in mammals, entering cells through amino acid transporters, eliciting oxidative stress, leading to altered gene expression and dopamine (DA) neurotransmission. Studies utilizing GFP-expressing transgenic *C. elegans* can directly investigate the role of specific proteins in neuronal function by assaying for neuron-specific morphology and behaviors. Using *C. elegans* expressing MCherry under the promoter for the DA transporter, we have found that MeHg alters cellular nicotinamide adenine dinucleotide ($NAD^+$) levels leading to both altered dopaminergic (DAergic) cell morphology and behavior as measured by the basal slowing response. Supplementing the worms with excess $NAD^+$attenuates the effects of MeHg on both behavior and DAergic cell morphology. Alterations of cellular $NAD^+$by MeHg may have profound affects on cells due to the inhibition of sirtuins, NAD-dependent enzymes involved in aging and epigenetics. Supported in part by a grant from the National Institute of Environmental Health Sciences (NIEHS) R01 ES07331.

**572 Abnormal gene expression leading to neurobehavioral abnormalities is induced by methylmercury exposure of grandparents**

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Methylmercury (MeHg) is a ubiquitous environmental neurotoxicant. Developmental exposure of zebrafish to MeHg is known to alter their neurobehavior in a manner similar to other vertebrates. The current study investigated the direct and transgenerational effects of MeHg, at tissue doses similar to those detected in exposed human populations, on sperm epimutations (i.e., differential DNA methylation regions [DMRs]), neurobehavior (i.e., visual startle and spontaneous locomotion), and transcriptomics in zebrafish, an established human health model. F0 generation embryos were exposed to MeHg (0, 1, 3, 10, 30, and 100 nM) for 24 hours ex vivo. F0 generation control and MeHg-exposed lineages were reared to adults and bred to yield the F1 generation, which was subsequently bred to the F2 generation. Direct exposure (F0 generation) and transgenerational actions (F2 generation) were then evaluated. Hyperactivity and visual deficit were observed in the unexposed descendants (F2 generation) of the MeHg-exposed lineage compared to control. An increase in F2 generation sperm epimutations was observed relative to the F0 generation. Investigation of the DMRs in the F2 generation MeHg-exposed lineage sperm revealed associated genes in the neuroactive ligand-receptor interaction and actin-cytoskeleton pathways being effected, which correlate to the observed neurobehavioral phenotypes. Developmental MeHg-induced epigenetic transgenerational inheritance of abnormal neurobehavior is correlated with gene expression changes and sperm epimutations in F2 generation adult zebrafish. Therefore, mercury has the ability to promote the epigenetic transgenerational inheritance of disease in zebrafish, which significantly impacts its environmental and ecological health considerations. Further study of the transgenerational health effects of MeHg is required to better understand the risks for exposed populations, which, if transgenerational effects are ignored, are dramatically underestimated.

## Plants in Environmental Risk Assessment: Assessing and Predicting the Effects of Chemicals on Plant Communities

**573 A field study method as a potential higher tier option to refine risk assessment for non-target terrestrial plants**

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During the regulatory approval process for pesticides, potential side-effects to non-target terrestrial plants (NTTP) are assessed. Routine testing with NTTPs is done in standardized greenhouse studies under conservative assumptions. If the obtained results show a concern for NTTPs, higher tier testing may be needed to assess whether this concern may occur under more realistic conditions in the field. So far no agreed NTTP field study method has been established. Based on three pilot studies (2014-2016), we developed a method for conducting higher tier field studies with NTTPs using commercially available seed mixtures as the source for establishing study sites (posters SETAC Europe 2016&2017). In these studies, it was found that both seed mixture and growth area (including weather, soil etc.) greatly influence the distribution of species and homogeneity of replicates. Specific farming measures (e.g. sequential plowing prior to seeding) were defined to overcome the out-competition of the sown species by other species emerging from the soil seed bank. Here we present results of a field study performed in Germany during 2017 according to our established method. Effects of a sulfonyl-urea herbicide on 11 plant species sown as a seed mixture were evaluated throughout the growing season (May to September). Three herbicide rates plus a control were applied to four replicate plots each. Vegetation cover according to the Londo scale, dry biomass of total vegetation, symptoms of phytotoxicity as well as BBCH stage of the sown species were regularly assessed. Although the utilized farming measures reduced emergence of plant species from the soil seed bank, their occurrence seems to be unavoidable. Therefore, the spontaneously emerged species were included in the vegetation coverage and biomass assessments. The assessed endpoints and species showed different responses to the tested herbicide, e.g. we could estimate NOECs for coverage during early assessments, but not for biomass, while during posterior assessments, NOECs could be derived for biomass. As it is expected for a more realistic higher tier testing method, variability was high for some endpoints e.g. the treatments` coverage for *Borago officinalis* was in the control range at most assessments. Effects of the herbicide in field conditions were less pronounced than predicted by corresponding greenhouse studies confirming the suitability of the study for refining the risk assessment for NTTPs

**574 A comparison between vegetative and reproductive endpoints for two non target plant studies with non-crop species in greenhouse and field**

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Terrestrial plants are providing a broad spectrum of ecosystem services such as the provision of e.g. food and natural medicines or the regulation of air quality. Plants in the agricultural ecosystem can be divided into three basic groups: crop plants, target plants for herbicides (weeds) and non-target terrestrial plants (NTTPS). The only refuges for NTTPS in intensively used agricultural areas are often field margins representing the majority of semi-natural habitats. Since agriculture is the dominating land-use in the temperate zone protection of non-target plants is a regulatory aim. Using herbicides to reduce weed competition in agricultural areas can adversely affect non-target terrestrial plants growing at field margins.For a more realistic assessment of effects there are several suggestions to include also non-crop species in the testing scheme from the list provided in OECD guidelines (OECD 208 and 227) and to assess the life-cycle with flowering and seed production. In this work two separate studies are compared to find out if non-crop species tested in greenhouse and in a field result in a comparable outcome. The experimental design used in both studies consists of one control and four application rates with the same herbicide product. In the greenhouse (OECD 227) 6 replicates per rate were used while in the field 8 replicates were used to account for the higher variability in the field. Furthermore, in the greenhouse species were grown individually while the field study consisted of plant communities. The four rates included the field rate down to a 3% drift rate. Assessed were biomass and plant height at the vegetative and generative phase of the plants. The number of flowers and seeds and germination of the harvested seeds were assessed for selected species to evaluate differences in flowering and germination. Furthermore, in the field study effects on plant community were assessed with multivariate analysis. To make results more comparable MDD were calculated for the individual endpoints. Since the same rates and species are used it is possible to investigate the effects of pesticides to specific plant species in both scenarios and to confirm that a higher TIER approach is also useful for plants.

**575 Testing the emergent macrophyte, *Glyceria maxima* in a water-sediment system: Progress of ring-tests with Isoproturon and Imazapyr**

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Under EU pesticide regulation, regulatory tests are required for the aquatic macrophyte, *Lemna*, and two algal species for herbicides and plant growth regulators. Data requirements introduced under EU Directive 1107/2009 stipulate that further tests may be required for compounds which show selectively higher toxicity to either dicotyledonous or monocotyledonous plant species in terrestrial plant tests. In these cases, the recommended dicot and monocot species are *Myriophyllum* and *Glyceria*, respectively. OECD Test Guideline 239 for testing *Myriophyllum spicatum* in a water-sediment system was adapted to facilitate growth of the emergent, reed grass, *Glyceria maxima*, and ring-tested in 13 laboratories during 2016 and 2017 with the herbicide, isoproturon. Results from this ring-test were used to adapt the test protocol in terms of plant propagation recommendations, test system specification, test duration, assessment parameters and draft validity criteria. The revised protocol was then used in a second ring-test with the herbicide, imazapyr during the summer of 2018. Results of the isoproturon ring-test will be presented alongside progress on the second ring-test with imazapyr.

**576 Experimental Design and Model Selection for Ecotox Risk Assessment**

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Recent experience with regulatory requests for re-analysis of older studies using newer statistical methodology has resurrected an old statistical issue of designing a study to fit its objectives and the dangers of imposing a statistical structure on data not fit for purpose. There is a continual need to update statistical methodology as new ideas arise and software to implement these methods become available. Problems can arise when new methods are imposed on old experimental designs. Imagine buying a plot of land with a small cottage. If we tear down the cottage, but leave the cellar and foundation, and then build a mansion in its place but based on the existing foundation, the resulting structure can be unstable and severely restricted in functionality. This presentation will explore the relationship between experimental design and the type of statistical model that can be fit to the resulting data and endpoints that can be estimated or determined from the model. In some instances, newer methods can be applied without problem to existing data. In other cases, existing data cannot support newer methods. It is important to understand the data requirements of the methods or models we intend to use. The size effect that can be estimated or detected is critically important and is strongly related to experimental design and biological variability. There is a model underlying every statistical test used to derive a NOEC or estimate an ECx. The basic statistical model for a simple toxicity experiment is given by $Y_{ij}=\mu_i + e_{ij}$, where $\mu_i$ is the expected mean response in the $i^{th}$ concentration, and the $e_{ij}$ are independent random errors, usually assumed to be identically distributed. What distinguishes one model from another are what distribution is assumed for the errors or responses and what restrictions or assumptions are placed on the treatment means, $\mu_i$. It is possible to determine the size effect that can be estimated or detected from a given dataset and it depends largely on experimental design and response variability. Statistical models used for hypothesis testing or regression estimates have data requirements. Model assessment tools are well established and should be used in fitting models to ecotoxicity data. Ignoring these tools or model requirements can lead to poorly estimated effects and misleading results. Understanding these concepts enables the scientist to make sound assessments of the data collected.

**577 Biochar Enhances Seed Germination in a Legacy Mining Soil**

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Biochar, the carbon-rich material remaining after pyrolysis (heating in low oxygen atmosphere) of cellulosic feedstocks, has the potential as a soil amendment to sequester contaminants, improve soil water-holding capacity, and increase nutrient retention thereby enhancing soil conditions to benefit plant growth. Thus the benefits of biochar could enhance the establishment and growth of plants for mine site revegetation. We evaluated the potential for different biochar types and application rates on grassland species being considered for revegetation of land affected by historic lead and zinc mining in southwestern Missouri. We used a seed germination test to determine the effects of biochar on seed germination and early seedling growth for six plant species, as well as key soil characteristics, pH and EC for plants growing in a lead/zinc mine soil, with the lead-containing wastes removed. This study was a preliminary screening of plants growing on amended mine soil before full-fledged greenhouse and field studies. Plant species were *Andropogon gerardii* (ANGE, big bluestem), *Elymus Canadensis* (ELCA, Canada wildrye,), *Elymus virginicus* (ELVI, Virginia wildrye), *Panicum virgatum* (PAVI, switchgrass), *Schizachyrium scoparium* (SCSC, little bluestem), and *Sorghastrum nutans* (SONU, Indiangrass). Biochar was made from barnyard manure (BM), yard debris (YD), hardwood pellets (HP), cedar wood (CW) and prairie hay (PH) pyrolyzed at 350, 500 and 700 °C, and applied at rates of 1, 2.5 and 5% by weight. While there were few effects of biochar on seedling emergence, early seedling growth measured as dry weight was significantly increased by a number of the biochars. Species responded differently, as growth of PAVI and SONO seedlings growth was increased by nearly all biochars, ELCA and ELVI growth was increased by many biochars, and ANGE and SCSC growth was largely unaffected by biochar. Soil pH was increased by nearly all biochars, and soil extractable phosphorus (EP) was increased by many of the biochar treatments, but especially by the higher nutrient content BM and PH biochars. Soil electrical conductivity (EC) was increased primarily by BM and PH. Nearly all effects of biochar on pH, EP and EC were greatest at the 5% biochar application rate. This study indicated the potential for biochar to enhance early seedling growth in mine affected soils, largely by improving soil chemical characteristics.

**578 Microplastic exposure elicits responses of spring onions and associated soil microbiomes**

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Microplastics are among the most long-lasting contaminants, and this novel stressor might cause several ecological impacts on marine and fresh waters. However, there is a lack of information on the risks of this pollution on land. Thus, it is highly relevant to unravel potential effects of microplastics on processes of terrestrial function. We contaminated a loamy sand soil with 6 different types of microplastics (polyester fibers, polyamide beads, and 4 micro-fragment types: PE-HD, PET, PP, and PS), plus a control without plastic addition. Polyester fibers were added to 0.4% of soil weight, while other plastics were added to 2.0% of soil weight. Each treatment included 12 replicates of a single microplastic type mixture with soil. Soil was kept in glass beakers in the dark for 2 months at 90% water holding capacity for soil microbiome acclimation. Then, we introduced in half of experimental replicates (N=6) seedlings of *Allium fistulosum* (spring onion) that had been germinated under sterile conditions. After a 2-months growth period, the evapotranspiration rates were assessed. The spring onions were then harvested for measurement of plant biomass (above-ground, onion bulb, and roots), and root traits. We also quantified proxies of soil function (water stable aggregates, soil structure, and bulk density). Finally, we assessed microbiome diversity and function via soil microbial activity (FDA), root mycorrhizal colonization (root staining), and DNA sequencing of bulk soil and rhizosphere bacteria, fungi, and cercozoa. No significant lethal toxicity could be observed for this plant model in response to any of the plastics. However, diverse changes on plant-soil responses were observed in case of exposure to polyester fibers and polyamide beads. The fibers increased below-ground biomass (root and onion bulb), while beads increased 3-fold above-ground biomass and significantly decreased above-ground/ root ratios. These changes were accompanied by significant effects on soil biophysical environment, microbial activity, and microbiome diversity. Our unprecedented results highlight the potential of microplastics to trigger environmental change in terrestrial systems via mechanisms other than direct toxicity. Considering such effects and the potentially near-permanent widespread terrestrial contamination, ecologically significant baseline shifts caused by microplastics in soil systems and their plant holobionts cannot be ignored.

**579 F1 seed germination and seedling growth of rice (Oryza sativa japonica) exposed to arsenic and copper oxide nanoparticles**

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An F1 rice (*Oryza sativa japonica*) seed germination and seedling growth test was conducted to investigate the intergenerational effect between F1 and F0 with different treatments of arsenic (As) and copper oxide nanoparticles (nCuO). F0 rice seeds were exposed to As (0 and 10 mg/kg sand) and nCuO (0, 0.1, 1.0, 10, 50, 100 mg/L) in a whole life cycle greenhouse study. F1 seeds were harvested from the 12 treatments (2 levels of As × 6 level of nCuO) and exposed again to the same treatments as F0 seeds in addition to control and As alone treatment. F1 tests were conducted for 18 days in an incubator with temperature controlled at 25 ± 1 ? during daytime (16 h) and 20 ± 1? at night (8 h). F1 seed germination was influenced by treatments that F0 seeds received ($p < 0.05$). Seeds produced from treatment with nCuO at 50 mg/L had the lowest germination percentage ($p < 0.05$). Neither As nor nCuO exposure individually influenced F1 seed germination, but the interaction of As and nCuO differentiated the germination at various concentrations of nCuO ($p < 0.05$). F1 seedling shoot length (SL), root length (RL), and the number of root branches (NRB) were all affected by different F0 treatments ($p < 0.001$). The SL and RL of F1 seedlings were all decreased by F1 As alone treatment compared with control ($p < 0.001$), whereas the NRB was increased by F1 As alone treatment ($p < 0.001$). The F1 seedling SL was mainly decreased by F1 exposure to nCuO ($p < 0.001$). The interaction of As with nCuO at low concentrations (0, 0.1 and 1.0 mg/L) of nCuO decreased the F1 seedling SL, and increased by the interaction at high concentrations of nCuO (50 and 100 mg/L) ($p < 0.001$), corresponding to the same nCuO concentration without As. The F1 seedling RL were all decreased by As and nCuO, and the interaction of the two toxicants was significant ($p < 0.001$). The NRB was primarily increased by As treatment ($p < 0.01$). To conclude, transgenerational effects were observed on both germination and early seedling growth of rice. Additional respective exposures of F1 seeds to As or nCuO did not affect seed germination but affected the seedling growth. The interaction of As and nCuO was significant on seed germination, the SL, and RL.

**580 Translocation of [14C] Plant Protection Product in Tomato: Evaluation of Foliar and Soil Application by Autoradiography**

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Once applied to a plant, chemical residues have the potential to move to other plant tissues via phloem and xylem. This translocation can affect pollinating insects and consumers of the plant tissues. In this study the translocation of a plant protection product to various tomato tissues (flower, leave, stem, and root) was evaluated. Tomato plants of a common commercial variety at the BBCH 51 stage were split into three groups: an untreated control group, a foliar application group, and a soil application group. A suspension formulation of [$^{14}$C]active ingredient of a plant protection product was applied in a single application at a rate of 0.50 kg a.i./ha by foliar application and soil application to the corresponding groups. Leaves, stems, and flowers were harvested at 1, 2, 4, 6, and 8 weeks after application and root tissue was harvested at 8 weeks after application. At each sampling interval, the collected tissues were analyzed for total radioactive residue (TRR) by combustion analysis and autoradiography by phosphor-imager analysis. Select tissue samples were extracted and analyzed by HPLC with radiochemical detection (HPLC-RAM). The combustion results demonstrated that the TRR were higher in soil application tissues than in foliar application tissues for all tissue types at all sampling intervals. The autoradiographs were correspondingly darker in the soil application tissues. HPLC-RAM analysis indicated that the majority of extracted residues were metabolites of the active ingredient. These data indicate foliar application resulted in reduced translocation of this active ingredient of a plant protection product – and metabolites thereof - when contrasted with soil application. In conclusion, translocation can be effectively evaluated by autoradiography. This procedure can be incorporated into a conventional plant metabolism study, and can provide valuable information to better assess the potential exposure of pollinating insects and consumers to plant protection product residues.

## Bridging the Gap Between Toxicity Method Challenges and Regulatory Compliance for Effluent and Ambient Toxicity Programs

**581 NPDES permitting – How Permit Language Affects Laboratory Performance**

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National Pollutant Discharge Elimination System (NPDES) permitting regulations under the Clean Water Act (CWA) have been in place since the mid 1980's. As part of these regulations, Whole Effluent Toxicity (WET) testing has been utilized as a tool in NPDES permitting to protect the narrative water quality standards on receiving streams and prevent the discharge of toxics in toxic amounts. The science utilized in developing the WET methods is well understood in the laboratory setting; however, the transfer of technical knowledge, test nuances, method variability and the proper implementation of these methods, as prescribed in NPDES permits, is not consistent across the nation. WET laboratories, national laboratory accreditation programs, and regulatory staff have been working to standardize approaches but more work is needed. This session will provide the EPA NPDES permitting regulatory perspective on the inclusion of WET requirements in permits, the lack of specificity needed to control WET test requirements, and the troubles caused by minimal permitting controls. Additionally, regulatory resources and options will be discussed to aid in the advancement of the science from the public perspective as approached through the provisions provided under the CWA. *This session will provide direct implementation (DI) permitting experience as viewed by the EPA regional offices.*

**582 Update on the WET Method Errata and Data Questions for Whole Effluent Toxicity Test Methods**

*T.J. Norberg-King, USEPA / NHEERL / Mid-Continent Ecology Division*

The Clean Water Act sets the goal of maintaining the chemical, physical, and biological integrity of the Nation's waters and the national policy, and with that goal, the discharge of toxic pollutants in toxic amounts is prohibited. The use of aquatic toxicity testing, such as whole effluent toxicity (WET), under the National Pollutant Discharge Elimination System (NPDES) permits program, is an important component of the Environmental Protection Agency's integrated approach to water quality-based toxics control, along with aquatic life chemical-specific criteria and biological assessments. Over the years, continued toxicity testing method modifications, laboratory accreditation, increased training, and technical resources have abounded for toxicity tests with the widespread use of the promulgated methods. In whole effluent testing, the use of the toxicity test has become a valuable component of most water quality monitoring programs, and the use of standardized acute and sublethal freshwater and marine methods specify test acceptability criteria (TAC) for survival, growth, reproduction and provide specific testing procedures. Acute toxicity of effluents appears to have decreased since the WET testing has been required. Errata have been periodically published, and a summary of the errata will be presented. Data reviews of test results have shown laboratories interpret the required procedures differently. For example, while the time frame to end the chronic *Ceriodaphnia dubia* test is when 60% of the controls have three broods, some labs do not terminate until 80% have the third brood. Interpretation guidance is needed for these types of issues, and by strengthening the knowledge exchange between practicing laboratories, more consistent interpretations should be able to be made. Additional species are needed for effluent assessments, and methods are being assessed for two sensitive species, e.g., mayflies and mussels. This presentation will discuss the progress that has been made in the conduct and application of these tests and changes made in EPAs errata, along with recommendations to improve the quality and utility these tests, including suggestions for laboratory proficiency guidelines. *This abstract does not necessarily represent the position or policy of the USEPA.*

**583 Improving the Laboratory Accreditation Process for WET Methods: Progress of the NELAC's Whole Effluent Toxicity (WET) Testing Expert Committee**

*T.J. Norberg-King, USEPA / NHEERL / Mid-Continent Ecology Division; R.B. Naddy, TRE Environmental Strategies; P.F. De Lisle, Coastal Bioanalysts Inc; E.G. Briggs, Bio-Analytical Laboratories, Inc.; E. West, Louisiana Environmental Laboratory Accreditation Program, LDEQ Northwest Regional Office / Technical; S. Rewa, Environmental Resources Management*

Fostering the generation of environmental data of known and documented quality through an open, inclusive, and transparent process that is responsive to the needs of the community is a mission of *The NELAC Institute* (TNI) with the National Environmental Laboratory Accreditation Program (NELAP) (www.nelac-institute.org). TNI envisions a national accreditation program, whereby all entities involved in the generation of environmental measurement data within the United States are accredited to one uniform, rigorous, and robust program that has been implemented consistently nationwide and focuses on the technical competence of the entity pursuing accreditation. With NELAP concensus standards of best professional practices, the quality and reliability of environmental data used by federal and state agencies will be improved. Many fields of accreditation are available: metals, nutrients, oxygen demands, semivolatile organic compounds, general chemistry I and/or II, microbiology, asbestos, synthetic organic chemicals, volatile organic compounds, radionuclides, whole effluent toxicity testing, hazardous waste characterization, petroleum hydrocarbons, perchlorate, and/or basic environmental laboratory. As such, the TNI Whole Effluent Toxicity (WET) Testing Expert Committee serves to update and maintain the whole effluent toxicity testing standard while providing technical assistance on issues related to whole effluent toxicity, develop tools to aid implementation, and to facilitate the implementation of the TNI Standard. The workgroup provides guidance for standardizing test conditions required for proficiency testing (PT)/ Discharge monitoring report quality assurance (DMRQA) studies performed under the auspices of the USEPA, rather than the current practice of conducting multiple tests using different NPDES permit test conditions, so that a statistically significant number of comparable sample results are available. Another recommendation was to improve the statistical power and evaluation of WET data sets and results in PT/DMRQA studies by selecting one statistical method to calculate the test endpoint and eliminating the use of hypothesis test endpoints. With experts, the programs for TNI on WET testing methods, quality control and data interpretation is updated. In this presentation, we will highlight some of the solutions to these issues as recommended by TNI WET workgroup. *This abstract does not necessarily represent the position or policy of TNI or the USEPA.*

**584 The Value and Challenges of WET as a Compliance Tool – Part II**

*R.B. Naddy, TRE Environmental Strategies; D.A. Pillard, TRE Environmental Strategies / Environmental Toxicology*

Why is it that an unfavorable analytical value is more acceptable by a permit-holder compared to an unfavorable whole effluent toxicity (WET) value which is often viewed with disbelief and doubt? In addition to a general mystery among some permit holders about the purpose of WET and how, or why, it applies to permit compliance, an oft-heard criticism of WET (even though it has been around for decades) is: there is very little if any progress or development of methods. This is in contrast to analytical methods, which appear to be more fluid. While being able to measure the concentration of a chemical in the environment is important, shouldn't equal importance apply to methods that assess whether that chemical is bioavailable to organisms in the environment? In this paper we will present numerous factors that contribute to the skepticism some have for WET. These include accuracy and precision of test data (e.g., how repeatable and useable are these data), representativeness of test species (there are few species and test methods), and regulatory application of WET results (what constitutes a 'toxicant'?) to name a few. Among the discussion points presented is the ultimate need for all interested parties to cooperatively participate in actions that will help address some of these WET shortcomings, and further refine the WET tool to its fullest potential. Only then will we be able to bridge the gap between WET method challenges and regulatory compliance.

**585 Strategies for ecotoxicology assessment of physical, biological, and chemical stressors in ambient waters**

*W.L. Goodfellow, Exponent, Inc. / Ecological and Biological Sciences Practice; S. Paulsen, Exponent Inc. / Environmental and Earth Sciences*

Compliance parameters for NPDES permits typically employ a suite of physical, chemical, and ecotoxicological tests to determine whether an effluent discharge has an adverse impact to the receiving waterbody. Traditionally, acute and chronic toxicity testing have been used for assessing whole effluent toxicity (WET) to fishes, invertebrates, and plant surrogate test organisms in laboratory exposures using prescriptive methods. However, addressing impacts observed in the environment often requires consideration of physical stressors, biological toxins (e.g., harmful algae blooms), and chemical stressors in ambient waters and sediments and thus often requires non-traditional tests or the application of traditional tests in non-traditional manners. This presentation will provide strategies that have been used successfully to assess the impacts of physical, biological, and chemical stressors in ambient waters and sediments. These strategies focus on determining causation of a specific stressor(s) and use of a weight-of-evidence approach in field and laboratory assessments of ambient waters and sediments.

**586 *C. dubia* reproduction impairment in low conductivity culture water**

*M. Vasi Stillway, University of California, Davis / VMAPC; C.A. Irvine, RBI / Ecosystem Services; S. Teh, University of California, Davis / School of Veterinary Medicine*

The chronic toxicity test method for *Ceriodaphnia dubia* is widely used for ambient surface water toxicity testing, regulatory compliance, or other programs. The USEPA test methods (i.e., method 1002.0) are relatively clear; however, interpretation of results is less clear when secondary controls are included as part of testing to account for background water quality related responses. In our study, monthly ambient surface water samples from the Sacramento-San Joaquin Delta, California, were collected from multiple locations in 2015, 2016, and 2017 as part of a regional monitoring program. These samples were characterized with short-term chronic toxicity testing and current use pesticide analyses. Toxicity tests included low electrical conductivity (EC) controls when ambient samples had specific conductance below 100 uS/cm. *C. dubia* reproduction in the lab culture water controls averaged 28.6 neonates/female while reproduction in the low-EC controls averaged 20.8. Reproduction in the low-EC controls was significantly lower from that in the standard culture water controls in 12 of the 22 tests, with low-EC effects observed in conductivities up to 127 uS/cm. Although there is a lack of guidance in use of secondary controls, USEPA (2000) describes the need to include a concurrent dilution control (i.e., secondary control) when the dilution water differs from the water used to culture the test organisms (i.e., primary control). The sample result should be compared to the secondary control result when the two controls responses are not significantly different from each other. In this light, it may be appropriate to evaluate the test organism response relative to the control with water quality most similar to the tested sample, depending on program objectives. However, the USEPA does not offer guidance on evaluating samples when there is a significant difference between the primary and secondary controls. This may indicate that the secondary control responses were outside of the test organism tolerance, that acclimation of the test organisms may be necessary or a different species may be more appropriate for use in these low conductivity ranges, or that project managers may wish to reevaluate test objectives. This presentation will share data and potential test interpretations that can be made, and will provide suggestion on how to interpret tests when using dual controls for ambient water testing programs.

**587 Method development for conducting effluent tests with the mayfly Neocloeon triangulifer**

*D.J. Soucek, Illinois Natural History Survey; A. Dickinson, Illinois Natural History Survey / Ecology; T.J. Norberg-King, USEPA / NHEERL / Mid-Continent Ecology Division*

Currently, USEPA relies on three freshwater species for short-term toxicity testing in a variety of programs and all tests are 8-d in duration or less. These methods are published in USEPA's Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. The methods use a fish, a cladoceran, and an alga, but to date, there have been no EPA-approved methods to use sensitive invertebrates like mayflies, which are among the most sensitive species tested with metals and major ions, to support NPDES permit decision-making (i.e., via incorporation of whole effluent toxicity (WET) tests). Methods for conducting acute 4-day and full-life chronic (~25-30-d) toxicity tests with the mayfly (*Neocloeon triangulifer*) have been published, but there is a need to extend the methodology so that it is applicable for testing effluents and receiving waters in a short-term exposure (e.g., 7 or 10 d). Our studies involved identifying an optimal starting age, test duration, and sub-lethal endpoint for WET testing. Others have compared sensitivity of this species at 0, 3, and 5 days-old, and we sought to further investigate this question with independent experiments comparing 0 and 7 day old organisms in 7 and 14-d tests. We also developed a length versus dry weight relationship for this species with the idea that while dry weight is a more sensitive endpoint than length, length is much easier to consistently and accurately measure with young instars of this species. The other objective of this study was to investigate and further refine various aspects of diatom culture technique on food quality and therefore mayfly growth. Optimizing diet for these organisms may be critical for achieving consistently high growth rates with low intra-treatment variability. Results of the study will provide further data needed to guide the development of a whole effluent toxicity test method for mayflies. *This abstract does not necessarily represent the position or policy of the USEPA.*

**588 Method Development for Conducting Short-Term Effluent Tests with Freshwater Mussel (Fatmucket, Lampsilis siliquoidea)**

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Short-term (6-8 d) freshwater toxicity test methods have been promulgated by the USEPA for estimating chronic toxicity of effluent and receiving water. These methods include a fish (*Pimephales promelas)*, a cladoceran (*Ceriodaphnia dubia)*, and a green alga (*Raphidocelis subcapitata*). Recent studies have demonstrated that a unionid mussel (fatmucket, *Lampsilis siliquoidea*) is one of the more sensitive species for ammonia, some metals, and major ion salts. Therefore, use of mussels as an additional species holds promise for effluent toxicity assessments. The objective of this study was to develop standard effluent test methods with fatmucket by, initially, determining optimum feeding rates and starting ages of juvenile mussels (~1-, 2-, and 3-wk old) in a 7- and 10-d feeding study; afterwards, assessing the sensitivity of the three ages of mussels in 7- and 10-d NaCl toxicity tests; and finally evaluating the performance and variability in the mussel methods through an interlaboratory study. For the feeding study, an algal mixture (~510 nl cell volume/mL) was prepared by mixing a commercially available algal concentrate and shellfish diet in test water (diluted well water at a hardness of 100 mg/L $CaCO_3$). The mussels were fed at four rates in the feeding study: adding 1, 2, or 3 mL of algal mixture twice daily or 4 mL once daily into each replicate chamber containing 200 mL of water. Mean survival was ≥93% in all feeding treatments except for the 4 mL fed once a day to 1-wk-old mussels (78%). The increase of mussel shell length ranged from 24 to 52% at test day 7 and from 28 to 60% at test day 10 among the four feeding treatments. The best growths (length) were observed at the feeding rate of 2 mL twice daily in treatments started with 1- to 2-wk-old mussels (~0.3 to 0.4 mm length), and at a higher feeding rate of 3 mL 2 times daily for 3-wk-old mussels (~0.5 mm length). The optimum feeding rates were used in the 7- and 10-d NaCl toxicity tests started with the 1-, 2-, or 3-wk-old mussels. For both test durations, the growth was consistently more sensitive than survival, and the sensitivity to NaCl was essentially the same among the three ages of mussels. An interlaboratory study has been organized and will be performed in thirteen volunteer laboratories from the United States and Canada, using 1-wk-old fatmucket in a 7-d NaCl exposure. *Disclaimer: This presentation does not necessarily reflect the views or the policies of the U.S. Environmental Protection Agency*

## Chemical Prioritization Using 21st Century Science: Considerations for New Approach Methodologies

**589 New Approach Methodologies--Fools Gold or the Mother Lode?**

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New approach methodologies (NAMs) offer a more rapid and inexpensive means for development of relevant information to understand potential environmental risks of chemicals in products. The United States Environmental Protection Agency (EPA) in its recent Draft Strategic Plan to Promote the Development and Implementation of Alternative Test Methods expanded the concept of NAMs to include tools for chemical characterization; hazard identification and characterization; dosimetry and in vitro-in vivo extrapolation (IVIVE); and characterization of exposures to humans and the environment. EPA's vision of NAMs may be driven by the fact that its regulatory programs for chemicals in commerce are evolving due to recent reform of the Toxic Substances Control Act (TSCA), which requires EPA to prioritize and evaluate the risks of the thousands of chemicals on the TSCA Inventory. However, the need for expanded capacity and for more efficient and effective tools for prioritization and risk assessment of chemicals is not unique to the United States; the European Chemical Agency and the Canadian government also have significant programs applying NAMs. We provide an industry perspective regarding opportunities to reduce the use of vertebrate animals in toxicity testing and deliver greater value to industry, regulators, and stakeholders through chemical prioritization and ecological risk assessments that can be developed faster, at reduced cost, and with reduced uncertainty. In addition, we explore NAMs that go beyond replacing vertebrate animal testing, such as those that utilize in silico tools (e.g., QSPR, QSAR), existing data through read-across techniques, and existing high-throughput in vitro assay data incorporated into weight-of-evidence screening, prioritization, and assessment. Emphasis is placed on the processes needed to assure regulatory acceptance of NAM data, including critical engagement of industry, regulators, and other key stakeholders.

**590 Evaluation of Three Interventions to Improve Design and Safety of Industrial Chemicals**

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Despite their ubiquity in modern life, industrial chemicals are poorly regulated in the United States. Statutory law defines industrial chemicals as chemicals that are not foods, drugs, cosmetics, nor pesticides, but may be used in consumer products, and this distinction places them under the purview of the Toxic Substances Control Act (TSCA). The Frank R. Lautenberg Chemical Safety for the 21st Century Act addresses many but not all of TSCA's failings, and rightfully emphasizes the development and adoption of high throughput screens, in vitro, and alternative assays to improve the process for registering new chemicals and to address the tens of thousands of untested chemicals currently in the TSCA inventory. As the discipline of toxicology gradually shifts from its' history as a reactive science (responding to problems after they've occurred) to a proactive science (attempting to predict and circumvent dangers to human and environmental health), two things become clear: 1.) traditional low throughput toxicological testing methodologies are inadequate to address both the volume of chemicals of interest and the pace of research; and 2.) the modern industrial chemical ecosystem is complex and no single testing solution will be appropriate for all the actors that populate that ecosystem. To address these challenges, three interventions were investigated, each of which targets a different population within the industrial chemical ecosystem. The first intervention was a suite of computational toxicology methods targeted towards chemists in the initial phases of chemical design and development. The second intervention was an alternative assay, the yeast functional toxicogenomic assay, permits industrial or government labs to rapidly evaluate differences in cytotoxic mechanism between different chemicals – even if they are structurally very similar. The third intervention explored was a method for enhancing the metabolic capacity of cell lines currently used by regulators for high throughput cytotoxicity testing. These interventions individually are not necessarily appropriate for all actors across the US industrial chemicals ecosystem, but as bespoke solutions they may be quite useful, and, it is hoped, support a larger exploration of where and how similar efforts may be spent most effectively to reduce industrial chemical hazard.

**591 Can Alternative Toxicity Testing Methods Be More Efficient Than Traditional Methods?**

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Chemical contamination is considered to be one of the planet's greatest threats with numerous chemicals lacking basic toxicity information. Traditional animal-based toxicity testing involving characterizing apical measures (e.g., survival, growth, development) that has been the mainstay since the 1920s, is in the midst of transforming to an approach founded on a diverse range of new approach methods (NAMs). Such alternative testing methods are said to be faster, cheaper, and use fewer animals, however, there is little empirical evidence to support these notions. The objectives of this research were to A) examine the evolving field of environmental toxicity testing by outlining key events, challenges, and opportunities, B) synthesize available information on costs associated with money, time, and number of animals used, and C) present select case studies highlighting the potential benefits of using alternative approaches. Deliberate bibliometric searches were carried out for papers and reports detailing various costs associated with toxicity testing. We observed that events such as seminal publications on toxicity testing in the 21$^{st}$ century (TT21C) and the increasingly global threat of chemical contamination, and international legislations on chemical testing have highlighted the need for a paradigm shift in toxicity testing. Current challenges are manifold: there are 20,000 to 100,000 chemicals registered in commerce worldwide; concomitantly, there is an urgent need for faster and more resource efficient methods that can provide the type of data usable for regulatory purposes. Technological advancements including high throughput sequencing, toxicogenomics, bioinformatics, and adoption of the adverse outcome pathway framework along with large-scale efforts such as ToxCast can help realize the vision of TT21C in ecotoxicology by prioritizing 3-15% of chemicals that are estimated to need further animal testing. Comparisons between various toxicity tests indicate that whole animal tests tend to be more expensive than alternatives (median cost: \$118,000 vs \$2,500), are slower (20 weeks vs 3 weeks), and use more animals (130 vs 20). While the inherent experimental differences between traditional and alternative methods make it difficult to directly compare costs, select case studies such as the use of custom designed qPCR arrays and the endocrine disruptor screening program demonstrate that there is scope for the implementation of alternative methods.

**592 In silico computational transcriptomics reveals novel endocrine disruptors in Largemouth bass (Micropterus salmoides)**

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In recent years, decreases in fish populations have been attributed, in part, to the effect of environmental chemicals on ovarian development. To understand the underlying molecular events, we developed a dynamic model of ovary development linking gene transcription to key physiological endpoints, such as gonadosomatic index (GSI), plasma levels of estradiol (E2) and vitellogenin (VTG), in largemouth bass (LMB) (*Micropterus salmoides*). By mapping the responses of a transcriptome from LMB collected from a polluted site, we were then able to identify modules (clusters of genes), which are perturbed at different stages of ovarian development. A sub-network was identified that closely linked

gene expression and physiological endpoints and by interrogating the Comparative Toxicogenomic Database (CTD), Quercetin and Tretinoin (ATRA) were identified as two potential candidates that were associated to the observed molecular response in LMB ovary following reproductive disruption. Predictions were validated by investigation of reproductive associated transcripts using qPCR in ovary and in the liver of both male and female largemouth bass treated after a single injection of Quercetin and Tretinoin (10 and 100 µg/Kg). Both compounds were found to significantly alter the expression of some of these genes. This study demonstrates that by utilizing computational approaches and online knowledge bases to understand the underlying molecular response of organisms, it is possible to identify putative chemical candidates that may impact reproductive health. This approach is highly relevant for classifying chemicals prior to conducting risk assessments, and we propose that this is a viable approach for chemical prioritization, reducing animal numbers, and developing safer chemicals in the public domain.

**593 Incorporating Lifecycle Emission Information in Promoting Chemical Exposure Screening**

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While high-throughput human chemical exposure screening often relies on hazard indicators such as persistence and bioaccumulation potential, realistic human chemical exposure and potential health risks are also dependent on factors such as chemical use information and chemical-specific exposure pathways. Multimedia, multi-pathway exposure models are being used for chemical prioritization and screening, the parameterization of which requires chemical specific use and emission information (e.g., how is it emitted? how much?). This information, however, is often insufficient or missing for most chemicals in commerce. To address this issue, we propose integrating substance flow analysis, which is a powerful tool to derive chemical emissions throughout the chemical/product lifecycle, into high-throughput screening for human exposure potential. As an illustrative example, we combine a substance flow model (a simplified version of CiP-CAFE) with chemical fate and exposure models (RAIDAR and RAIDAR-ICE), to create a modeling continuum from the quantity produced or imported to human aggregate exposure. Our model requires inputs of basic properties of chemicals (e.g., partitioning coefficients, and degradation/biotransformation rates) and associated products (e.g., use categories, lifespans and material characteristics). These properties can either be retrieved from existing data resources or calculated by models, which enables the model's wide applicability to high-throughput screening of both existing and new substances. The model allows "forward" evaluation of human aggregate exposure to chemicals arising from their actual use, or "back" calculation of the critical production/import quantities of a chemical that would not cause an unacceptable level of risk (the latter depends on the availability of toxicological data). Overall, the approach shows promise in high-throughput exposure screening of the myriad of chemicals.

**594 Application of the RAIDAR Model to Aid Chemical Prioritization in Canada**

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A common approach used in regulatory programs to evaluate chemicals for their potential effects on the environment is to categorize chemicals using various criteria for Persistence, Bioaccumulation and Toxicity (PBT). Alternative methods screen and prioritize chemicals based on estimates of exposure and potential risk. In the US, recent amendments to the Toxic Substances Control Act (TSCA) have led the United States Environmental Protection Agency (USEPA) to consider new approach methodologies (NAM) in many aspects of TSCA implementation including prioritization. Much focus has been directed towards in vitro toxicity testing. As highlighted in the National Academy of Sciences, Engineering and Medicine report "Using 21st Century Science to Improve Risk-Related Evaluations" there is a need to develop and evaluate exposure models to compare against existing and emerging data streams of toxicity information to guide risk-based decision-making. The Risk Assessment IDentification And Ranking (RAIDAR) model combines chemical fate and transport at a regional scale with bioaccumulation in aquatic, terrestrial and agricultural food webs using toxicokinetic models. RAIDAR simulated external and internal exposures to a range of ecological receptors and humans are quantitatively compared to toxicity data expressed in terms of external (e.g., LC50, TTC, or eco-TTC) or internal (e.g., critical body residues) effect or no effect concentrations in RAIDAR calculated Risk Assessment Factors (RAFs). The latest version of RAIDAR includes methods to better simulate the fate, bioaccumulation and exposure for ionogenic organic chemicals (IOCs). We applied the model to approximately 10,000 organic chemicals on the Canadian Domestic Substances List using various methods for characterizing chemical toxicity (i.e., LC50s, critical body residues and chemical activity) in a risk-based prioritization exercise. The results of RAIDAR modeling will be integrated into version 2.0 of ECCC's Ecological Risk Classification (ERC) approach which is being developed as one approach to inform chemical priorities for post 2020 chemicals management in Canada. The RAFs span approximately 10 orders of magnitude. The greatest overall source of uncertainty in the RAF is the emission rate followed by biotransformation half-life estimation and toxicity information. The results of the RAIDAR calculated RAFs for chemical prioritization are compared against PBT categorization results.

**595 3D-Cultured Avian Cells for Chemical Screening: Metabolic and Gene Expression Profiles of the Chicken LMH Cell Line**

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There has been an increased demand for in vitro toxicity testing in order to efficiently screen and prioritize the vast number of chemicals in the environment that require assessment. Primary cell culture approaches have been the most common in vitro model for toxicity testing and chemical screening in avian species. However, the preparation of primary cells is relatively time consuming and requires the use of many animals. The use of immortalized cell lines could eliminate these requirements. Furthermore, advances in 3D culturing techniques permit cells grown in vitro to have a more tissue-like metabolic and gene-expression response to external stressors. In this study we began to investigate if the immortalized chicken hepatocellular carcinoma cell line, LMH, could be a suitable alternative to primary chicken embryonic hepatocytes (CEH) when used in a well-established assay for chemical screening and prioritization. LMH cells were grown as two-dimensional (2D) monolayers (proliferating and confluent cells) and as three-dimensional (3D) spheroids. Cytochrome P450 (CYP1A4) activity and gene expression were compared between CEH and LMH grown in all three culture conditions following exposure to the dioxin-like compound 3,3',4,4',5-pentachlorobiphenyl (PCB126). CYP1A4 activity was measured using the EROD assay and changes in mRNA expression, associated with the aryl hydrocarbon receptor (AhR) pathway, were determined using a custom-designed PCR array. LMH cells only showed EROD induction when grown in 3D spheroids, although at a lower level than CEH. Similarly, the greatest numbers of AhR-related genes were induced in LMH 3D spheroids compared to proliferating and confluent cells. Overall, these results suggest that LMH cells grown as 3D spheroids have a metabolic and gene expression response that is comparable to CEH, and may make a suitable animal-free alternative for in vitro screening of chemicals.

**596 Pulling New Methodologies into Integrated Approaches to Testing and Assessment: A Chemical Industry Perspective**

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Chemical companies employ early screening methodologies during chemical research and development to screen out chemicals that show signs of unwanted toxicological hazards or a poor environmental fate profile. These early screens include a combination of in silico tools and in vitro assays that are designed around key events or indicators linked to adverse outcomes or undesirable environmental fate parameters (*e.g.* PBT, endocrine disruption, sensitization). The results from early screens can be used to guide subsequent testing, including in vivo evaluations which are required, in many cases, for chemical registration in different geographies. In order to provide guidance on the conduct and interpretation of data from diverse sources (*e.g.* phys/chem parameters, in silico*,* in vitro*,* and in vivo data) integrated approaches to testing and assessment (IATA) have been developed for endpoints that are critical for evaluating safety for human health and the environment. Several IATAs that have been developed within the chemical industry will be presented, including those associated with endocrine activity, bioaccumulation, and ecotoxicity. The role of new approach methodologies in these IATAs will be emphasized, and data gaps, where they exist, will be noted. Several chemical case studies and their evaluations in relevant IATAs will be presented with associated learnings and recommendations for future improvements.

## Current-Use Pesticides: Exposure and Effects on Non-Target Organisms and Ecosystems – Part 1

**597 Exposure and effects of clothianidin residues in corn pollen: Honey bee colony simulation in a field setting**

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As managed pollinator species, honey bees provide major pollination services to a wide variety of crops across the globe. At the same time, they are potentially vulnerable to the effects of systemic neonicotinoids because residues can occur in pollen and nectar collected by the bees. However, the assessment of potential effects of neonicotinoids on colonies in field studies is challenging because multiple environmental conditions interact with the colonies' health. Honey bee colony models such as BEEHAVE provide the opportunity to assess potential influx of residues into a colony via different routes, and their effects on bees in the hive can be dependent on their stage-dependent consumption rates and sensitivities. We extended BEEHAVE to represent exposure to clothianidin via residues in pollen from treated corn fields. Landscapes around the colonies were simulated using land cover data from sites across the Midwest of the United States. Simulated foragers collect pollen from flower resources across the landscape including corn pollen during the corn blooming period. Clothianidin residues are consumed by larvae and worker bees. Different residue levels in corn pollen were applied to assess impacts on honey bee colonies over a one-year cycle. Clothianidin effects on colony strength were only observed if unrealistically high residue levels in the pollen were simulated. The landscape composition significantly impacted the collection of pollen (residue exposure) from the corn fields, resulting in higher colony-level effects in landscapes with low proportions of semi-natural land. The case study with the mechanistic honey bee colony model presents a path to the application of such models in the context of pesticide risk assessment.

**598 Influence of flower resources and neonicotinoid exposure on bumble bee colony development and reproduction**

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Bees in agricultural landscapes are affected by numerous stressors and a key limiting factor is lack of flower resources. Mass-flowering crops can mitigate this stress, but such nectar and pollen resources are often contaminated with pesticides that can negatively affect bee abundance and reproduction. Our understanding of how the interplay between flower resources and pesticide exposure affects bee colonies foraging in real landscapes is, however, limited. To explore this, we placed three bumble bee (*Bombus terrestris*) colonies in each of 18 landscapes of three types. Six landscapes were centered on conventionally managed red clover seed fields treated with the neonicotinoid thicaloprid (flower resources + pesticide exposure), 6 landscapes were centered on organically managed red clover seed fields (flower resources) and 6 landscapes lacked red clover seed fields within a 2 km radius (control landscapes). We confirm that bumble bees foraging in red clover seed fields treated with thiacloprid to control seed predating weevils were temporarily exposed to high levels of thiacloprid through pollen. However, the exposure level declined sharply over a two week period after spraying. Bumble bee colonies grew larger in landscapes with thiacloprid treated red clover seed fields, compared to colonies in landscapes without red clover seed fields. Colony weights did not differ between landscapes with thiacloprid treated red clover seed fields and landscapes with organically managed, non-pesticide treated red clover seed fields, indicating that the red clover provides important resources that support colony growth even when treated with thiacloprid. Production of new queens was positively related to peak colony weight. Although queen production did not differ significantly between the three landscape types, reproduction was numerically lowest next to treated clover fields after taking into account that these colonies grew the largest. We conclude that thiacloprid for pest control in red clover seed production appears to be of low potential risk to bumble bees, although further studies are needed to exclude possible negative impacts on bee reproduction. We suggest that addition of flower resources in agricultural landscapes can be an option to support pollinators even if they contain residues of pesticides.

**599 Assessing native bee abundance and species richness in imidacloprid-treated soybean fields and surrounding field-margins**

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Unlike honeybees, numerous wild bee species nest belowground and in close proximity to cultivated fields and flower foraging areas. Although agricultural field-margins can serve as important bee habitat, these areas may also accumulate neonicotinoid insecticides via environmental transport processes (e.g., runoff events and dust migration during planting). However, few field studies have evaluated neonicotinoid impacts on wild pollinator communities, including solitary, ground-nesting bees (e.g., sweat bees, longhorn bees). To assess effects of neonicotinoid exposure on native bee abundance and species richness, we sampled 30 soybean fields on five conservation areas in north-central Missouri from pre-seeding through harvest in 2017. Following baseline data collection in 2016, soybean fields were cultivated using one of three treatments: imidacloprid-treated fields ($n$=10); untreated fields ($n$=10); and previously treated (2016) to untreated fields ($n$=10). At each site, we collected field and field-margin soils, flower heads from wildflowers and soybean crops, and native pollinators every 28 days over five sampling periods (pre-seeding, post-seeding, growing, soybean flowering, and harvest). Neonicotinoid residues were detected in field soils during all sampling periods (frequency: pre-seeding, 7%; post-seeding, 33%; growing, 23%; soybean flowering, 53%; and, harvest, 33%). However, neonicotinoids were infrequently detected in margin soils (< 8% frequency, overall) with no residues detected in flowers from field-margins plants or soybean crops during the 2017 experimental year. Compared to untreated or previously treated fields, native bee richness was significantly less in neonicotinoid-treated fields ($\beta$ = -0.24 ± 0.10, $P$ = 0.05). Abundance of the more common genus *Melissodes,* which is composed of both generalist and specialist bees, was significantly less in fields with increasing soil concentrations of imidacloprid ($\beta$ = -1.33 ± 0.36, $P$ = < 0.001; marginal $R^2$ = 0.39). Here, we present our preliminary findings and discuss how this research improves our understanding of neonicotinoid seed-treatment use on non-target native pollinator communities within agroecosystems.

**600 Colony Level Impacts of Chronic Clothianidin Exposure via Spiked Pollen in Honey Bee Hives**

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Clothianidin is a systemic insecticide that can be detected in the pollen and nectar of treated crops. In order to evaluate the chronic toxicity of this neonicotinoid to honey bee colonies via a pollen route of exposure, pollen patties were spiked with test material and provided to colonies over a six week period. Pollen traps were affixed to hives in order to minimize incoming foraged pollen and maximize the consumption of the treated diet. Two separate pilot studies were conducted in 2017 with measured treatment concentrations of 82, 372 and 1460 ppb in one study and 127, 339 and 991 ppb in the second study. All control and treatment hives were replicated 8 times for both studies. In the high treatments in both studies the presence of dead bees in front of hives indicated elevated mortality at these concentrations. Three weeks into the exposure period, numbers of adult bees were significantly lower in the high treatments compared to the controls. Other colony assessment parameters (e.g., honey, brood) were not significant at this time point; however, by the end of the six week exposure period all colony condition endpoints were significantly lower in the high treatment colonies. These differences persisted until the end of the studies which was two months after the end of the exposure period. Lower pollen patty consumption was observed at the 372 ppb treatment, but no other significant differences from the control were observed at this level. Residue sampling from bee bread and uncapped nectar in the hives indicated minimal movement of clothianidin from the treated pollen patties into the hive food stores. While some clothianidin detections occurred, none were above the limit of quantitation (1 ppb for uncapped nectar, 2 ppb for bee bread). A definitive, GLP colony feeding study with spiked pollen patties will run from May 2018 to April 2019 with nominal test levels of 100, 200, 400, 700, and 1000 ppb. Data generated in the first half of this third study will be presented alongside results of the first two studies to evaluate the consistency of the study conclusions.

**601 Imidacloprid Monitoring in Urban Surface Waters of California, USA**

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Imidacloprid is a neonicotinoid insecticide used to control fleas, wood infesting pests such as termites and ants, and agricultural insect pests. Although neonicotinoid insecticides have come under scrutiny as a contributing cause of pollinator bee colony collapses, they also are quite toxic to sensitive aquatic organisms. Imidacloprid has been frequently detected in agricultural runoff; however, less is known about its environmental fate in urban landscapes. To address this, in 2010 the California Department of Pesticide Regulation initiated statewide imidacloprid monitoring at two main urban site types: storm drain outfalls and receiving waters. In addition, two small residential-neighborhood constructed wetlands were monitored. Imidacloprid was detected in over half (58%) of the 615 samples collected through March 2018. Imidacloprid detections were more frequent, with higher concentrations: 1) in Southern California than in Northern California; 2) at storm drain outfalls than at receiving water sites, and 3) during first flush rain events than during subsequent winter storms or non-rain (dry season) periods. However, non-storm mass loading contributed to a significant percentage of annual mass loading. This work shows that imidacloprid is a common contaminant in urban surface water, especially in Southern California, where imidacloprid is frequently detected at potentially toxic concentrations to sensitive aquatic organisms (i.e., above USEPA aquatic life benchmarks). Constructed wetlands in residential neighborhoods were ineffective in mitigating imidacloprid runoff. Therefore, alternative mitigation practices will be needed to reduce imidacloprid contamination in urban surface waters.

**602 Effects of environmentally realistic concentrations of neonicotinoid insecticides on aquatic invertebrates at population and community-levels**

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Neonicotinoid insecticides represent 24 % of the global market, and their use is increasing globally. Among them, clothianidin, imidacloprid, and thiamethoxam are widely used systemic insecticides, but are also used for lawn and garden care, and pest control. Residential usage has been linked to the occurrence of toxic level of pesticides in urban water bodies. Neonicotinoids are highly soluble in water and persistent in soil, and even though they are not intended for use in water bodies, they may enter in the aquatic compartment via spray drift, runoff or leaching, and contribute to downstream aquatic toxicity. Neonicotinoids interfere with the insect nervous system, and exhibit very high selectivity for insect nicotinic acetylcholine receptors. Although insects appear to be the most sensitive, some studies have shown effects of neonicotinoids on the crustaceans *Ceriodaphnia dubia* and *Daphnia magna*. However, most of the studies focus on single-insecticide exposure and very little is known concerning the impact of neonicotinoid mixtures on the environment and their combined toxicity on invertebrate community. As a first step, we tested the effect of a mixture of imidacloprid, clothianidin and thiamethoxam at concentrations measured in the environment, on *C. dubia*, at the population level and under controlled conditions. Population growth rate $\lambda$ was lower under exposure to the neonicotinoid mixture than in controls. Simulations performed using demographic stochasticity showed a higher risk to population extinction on mixture-exposed organisms (minimum abundance = 325, extinction risk = 0.07) in comparison to control (minimum abundance = 1208, extinction risk = none) and the mixture-exposed population never reached the carrying capacity (K = 500,000 individuals). Since neonicotinoid contamination is likely to induce a top-down trophic cascade in a community dominated by invertebrate predators, we then ran an outdoor mesocosm experiment to test the effect of the neonicotinoid mixture on an aquatic invertebrate community and to explore community-level effects. The community was sampled before the insecticide application and throughout the month following the treatment. Results will be discussed during the presentation.

**603 A field study assessing direct application of neonicotinoid seed treatment in wetlands: Implications for aquatic invertebrate communities**

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Neonicotinoid insecticides (NI) are commonly used as seed-treatments on major agricultural row crops (e.g., corn). Indeed, neonicotinoid treated agricultural crops are often planted directly in flood-plain wetlands managed for wildlife. Numerous studies have documented impacts of neonicotinoids to aquatic invertebrates in laboratory and mesocosm settings; however, less is known about impacts of neonicotinoids in field settings. We investigated invertebrate community response to planting of neonicotinoid-treated seed in managed wetland ecosystems. In 2016, we sampled water, sediment, and aquatic invertebrates from 22 paired wetlands on nine conservation areas during spring (pre-wetland draw-down) and fall (post-wetland flood-up) followed by a third sampling period (spring 2017, one year post-treatment). During summer, portions of sampled wetlands were planted with either untreated corn (control) or neonicotinoid-treated corn (thiamethoxam). Water quality parameters and concentrations of pesticides were used to evaluate effects of treatment on aquatic macroinvertebrate diversity and abundance using a series of generalized linear mixed effects models. Water and sediment concentrations of the six most common neonicotinoids were used to calculate overall NI toxicity equivalents (NI-EQs) based on an additive model of NI toxic equivalency factors (NI-TEFs: Cavallaro et al. 2017). Total mean NI-EQs for sediment (0.60 µg/Kg) were an order of magnitude greater than water (0.02 µg/L). Sediment neonicotinoid NI-EQs were greater in treated than untreated wetlands, both post-treatment (1.13 treated, 0.35 untreated, $p < 0.001$) and in spring 2017 (1.02, 0.28, $p < 0.001$). Water NI-EQs displayed similar results post-treatment (0.022 treated, 0.021 untreated, $p = 0.005$) and spring 2017 (0.014, 0.007, $p < 0.001$). Preliminary results indicate an overall decrease in aquatic invertebrate diversity and abundance with increasing concentrations of neonicotinoids in wetland water and sediment. Additionally, treated wetlands were observed to have lower invertebrate diversity and abundance compared to untreated wetlands post-treatment, but a recovery in abundance and diversity followed in spring 2017. Our results have implications for aquatic invertebrates as well as wetland-dependant species (e.g., waterfowl) as neonicotinoids concentrations, although below regulatory limits, are impacting these wetland ecosystems.

### 604 Invasive cane toads: Good for something? Developmental assays reveal safety concerns of the common rice herbicide, butachlor

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The intensification of rice agriculture and the corresponding increase in pesticide use poses a threat to biodiversity and human health. Amphibian species represent valuable indicators within rice ecosystems because they utilize them as habitat in the absence of natural wetlands, and pesticide applications often coincide with reproductive and developmental cycles. We conducted an experiment with wild cane toad (*Rhinella marina*) tadpoles at the International Rice Research Institute (IRRI) in the Philippines to 1) test whether environmentally relevant exposure to butachlor, an acetanilide herbicide used extensively in rice fields throughout Southeast Asia, affects development, behavior, thyroid physiology, and gene expression, and, 2) determine which developmental stages are most vulnerable to exposure, and 3) test the degree to which tadpoles are able to acclimatize to sub-lethal exposure. Our analysis revealed that *R. marina* exposed to butachlor developed slower, were less active, and weighed less, and that some concentrations affected the development of the thyroid gland: exposed individuals had fewer thyroid follicles and smaller thyrocyte cells. Furthermore, we found that *R. marina* eggs were more sensitive to exposure than tadpoles, and we observed compelling evidence of acclimatization: animals exposed to butachlor early in life performed better than naïve animals during a second exposure. Our findings support recent work indicating that butachlor causes thyroid endocrine disruption in vertebrates, and suggest that realistic exposure in rice fields presents a concern for wildlife populations and human health. Furthermore, because *R. marina* is a widespread invasive species and abundant within rice fields in the Philippines, we suggest using it as an indicator there, and in other tropical countries where it is native or has been introduced, to assess consequences of pesticide exposure.

## Adverse Effects of Chemicals on the Microbiome

### 605 Spatial and Temporal Shifts in Sediment Bacterial Community Composition Affected by Acidic Rock Drainage in the Animas River Watershed

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Acidic rock drainage (ARD) is a major source of metal pollution in aquatic ecosystems and is often associated with active and abandoned mines and mining-related activities. In Colorado alone, 230 hard-rock mines are known to release significant amounts of ARD. The Bonita Peak Superfund Site consists of 48 historic mining-related sources of ARD in southwestern Colorado which have heavily impacted the Animas River watershed for decades. Continual releases of ARD from the 48 sites compounded with natural weathering leaves several creeks on the headwaters of the Animas River with low pH and high concentrations of several toxic metals. As the metals flow downstream, they are sequestered in organic matter and settle in the sediment, creating a concentration gradient along the Animas River. Additionally, several 'blowout' events have been recorded, the latest on August 5, 2015, furthering the contamination of this watershed. Samples were collected in February 2015 and 2018. Thus, both temporal and spatial changes in bacterial communities downstream of ARD inputs in the watershed were examined. Further, given the timing of samples, we were able to compare the watershed after a blowout event to a watershed impacted by chronic, low-level releases of ARD. Closer to the Superfund site, chemotrophic extremophilic bacterial genera were identified. Specifically, we observed a high abundance of the genus *Gallionella* in our highly ARD polluted samples. Additionally, several other identified genera are thought to aid in the natural remediation of ARD. These genera may be suitable biomarkers of ARD contamination, or metal contamination from natural weathering, and could influence the remediation processes in these areas. Further down the concentration gradient, sediment microbiomes return to community structures more similar to those from non-impacted watersheds which are dominated by *Cyanobacterium, Rhodoferax,* and *Flavobacterium*.

### 606 The effect of nickel-contaminated sediments on the coral microbiome

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The mining and production of nickel has recently intensified in the Asia-Pacific region. As a consequence, large quantities of particulate-bound nickel will be transported to the coast as land run-off, potentially placing marine ecosystems, including coral reefs, at risk of adverse effects related to increased nickel and suspended sediment exposure. Coral reefs throughout Asia-Pacific are facing enormous threats of decline due to anthropogenic climate change, physical destruction, overfishing, pollution and sedimentation. These threats are exacerbated by the limited information on the impact of mining activities on coral reefs and tropical marine biota. This study used experimental facilities at the National Sea Simulator, Townsville, Australia to investigate the response of the common branching coral, *Acropora muricata*, to nickel-contaminated suspended sediments. To separate the physical effects from the sediment and the chemical effects from nickel exposure, simultaneously treatments were performed which included dissolved nickel (seawater only), clean sediments, and nickel-contaminated sediments. The experimental design consisted of 9 treatments: nickel in seawater-only, clean sediments (~ 4mg Ni/kg), laboratory-spiked nickel sediments (~ 6000 mg Ni/kg), and a field-collected nickel-contaminated sediment (~ 240 mg Ni/kg). All sediments were tested at low and high suspended sediment concentrations (5 and 30 mg/L TSS). The biological effects observed included bleaching, *Symbiodinium sp.* density changes, metal accumulation and assessment of the microbiota community structure (using DNA metabarcoding). Coral fragments (4-10 cm) were acclimated to laboratory conditions for ~ 2 months and then exposed to flow-through treatment conditions for 7 days. Samples were taken on days 0, 4 and 7 to measure coral health and to preserve tissues for DNA extraction. After 7 days the exposure was ceased and the corals were allowed to recover in filtered natural seawater for a further 7 days, after which all parameters were measured again. We found that low concentrations of sediment-bound nickel (≤ 240 mg/kg) did not negatively impact coral health but high sediment-bound nickel concentrations in combination with a dissolved nickel exposure induced significant negative effects to coral health. The effects of nickel-contaminated sediments on the coral microbiome will also be discussed.

**607 Life-stage, and species-specific effects of dietary methylmercury exposure**

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Mercury is a globally distributed contaminant, which is found even in remote aquatic ecosystems as a result of atmospheric deposition. Methylmercury, resulting from microbial transformation of inorganic mercury, bioaccumulates and biomagnifies in biota, resulting in potentially toxic body burdens in long-lived organisms at the top of aquatic food webs. Methylmercury can be actively transferred from mother to offspring, with the potential to cause severe, irreversible effects on developing organisms. Here, we describe the developmental effects of exposure to maternally-transferred dietary MeHg on a model fish species *(Pimephales promelas)*. Exposure to environmentally relevant concentrations of MeHg during development led to alterations in the dopaminergic system, metabolome, gene expression, behavior, hatch time, size, and embryo-larval survival. Similarly, effects on the dopaminergic system in specific regions of the adult *P. promelas* brain were observed after a 30-day dietary exposure. Recently, a functional link between gut microbiota and dopamine production in teleosts has been established. Therefore, we characterized MeHg-mediated changes to the gut microbiome composition in *P. promelas* adults. Because the dopaminergic system is highly conserved among taxa, we sought to confirm the altered dopamine concentrations in *P. promelas* brainin a higher vertebrate species. Metabolomics was performed on the mid-brains of male mice *(Mus musculus CD-1)*exposed to similar concentrations of dietary MeHg for 30-days. Changes in dopamine concentrations of the teleost brain were mirrored in the mid-brains of male mice, and several other significant changes to the mouse mid-brain metabolome were detected. Collectively, these results suggest current environmental exposure scenarios to MeHg are sufficient to induce a number of molecular-level changes that are associated with costs to whole organism fitness, with consequences for multiple life stages, and species. Due to the similar changes detected in mice, there is increasing evidence to suggest teleosts as a surrogate model species for studies assessing effects of MeHg on highly conserved systems.

**608 Shifts of microbiota of fish guts following exposure to dietary benzo[a]pyrene**

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Microbiota of animal guts have been described as an additional host ‘organ’ with well-established beneficial roles. However, little is known about the impact of a chemical exposure on both structure and function of gut microbiota of fishes. In order to determine the structural and functional shifts of gut microbiota of fathead minnows (*Pimephales promelas*) to chemical toxicants, juvenile fathead minnows were exposed to benzo[*a*]pyrene (BaP), a model compound simulating the effects of oil spills, at concentrations of 1, 10, 100, or 1000 µg/g, in addition to a vehicle control, for two weeks. Comparisons between multiple responses of gut microbiota, intestine, liver, and lymphocytes allow for discovery of an early warning response within exposed organisms. Overall and active compositions of gut microbiota will be characterized by use of DNA- and RNA-based 16s rRNA gene metagenetics, respectively. Expression levels of bacterial genes related with BaP degradation will be determined by RT-qPCR as well. Changes of several key genes related to BaP-exposure and immunotoxicity in gene transcript level will be determined by use of RT-qPCR with RNA isolated from intestines and livers from the hosts. Flow cytometry was used to determine shifts in lymphocyte proportions resulting from exposure. This research will provide preliminary information about the impact a controlled exposure may have on the interplay between host and bacteria, a lesser-understood component of environmental toxicity.

**609 A Comparative Analysis of Gill and Gut Microbiomes Across Fish Species Exposed to Oil, a Pathogen, or Both**

*M.L. Rodgers, R.J. Griffitt, University of Southern Mississippi / Coastal Sciences*

The microbiome is a complex assemblage of microorganisms that play an important, but poorly understood, role in mediating organism:environment interactions. Due to the complexity of the microbial assemblage, we are only beginning to understand how alterations to the microbiome may affect the host organism and its response to the exterior environment. Some research examining how the microbiome of fish exposed to xenobiotics is altered by that exposure has been done, but relatively little is known about how consistent or conserved induced alterations are. In order to understand how the microbiome varies across fish species with exposure to oil, exposure to a pathogen, and both simultaneously, juvenile red snapper (*Lutjanus campechanus*), Atlantic croaker (*Micropogonias undulatus*), red drum (*Sciaenops ocellatus*), and Southern flounder (*Paralichthys lethostigma*) were utilized. Fish were either exposed to high energy water accommodated fractions of oil (snapper, croaker, and drum), or oil mixed into sediment (flounder). All fish were exposed to oil for 7 days, then exposed to the known fish pathogen *Vibrio anguillarum* for one hour, followed by an additional 24 hours of oil exposure (8 days total). There were four experimental groups for each species: Control (no oil or pathogen exposure), Pathogen only (only exposed to *V. anguillarum* and not oil), Oil only (exposed to oil but not *V.* anguillarum), and Oil+Pathogen. Gill and gut tissues were harvested from all fish and microbiome analysis was performed for each sample. In Southern flounder, we found that in fish challenged with *V. anguillarum* in addition to oil, *V. anguillarum* accounted for nearly 40% of the gill microbiome and 20% of the gut microbiome, while all other groups (oil only, pathogen only, and control) had almost no representation from this species. In addition, flounder exposed to oil (regardless of whether or not they were also exposed to *V. anguillarum*) showed ~20% representation from *Alcanivorax* spp. in the gill microbiome, whereas this genus was not well-represented in control or pathogen-only groups. Finally, oil exposed versus non-oil exposed groups showed taxonomically distinct bacterial community assemblages, with gill and gut communities showing distinctions from one another.

**610 Effects of diet, taxonomy, and habitat on the gut microbiota of aquatic invertebrates**

*S. Kroetsch, University of New Brunswick Saint John / Biology and Canadian Rivers Institute; K.A. Kidd, McMaster University / Biology and Canadian Rivers Institute; S. Pavey, University of New Brunswick Saint John / Biology and Canadian Rivers Institute*

Most animals possess an internal and external community of bacterial microorganisms referred to as an organism's microbiome, of which the largest is present within the digestive tract. Whereas there has been considerable research linking human health to their microbiome, much less is known for aquatic organisms including macroinvertebrates. Before one can assess the effects of anthropogenic stressors on the microbiome, it is critical to develop an understanding of whether feeding habits and habitat affect the presence of gut bacteria. The current study assessed whether functional feeding groups, taxonomy or aquatic habitat characteristics impacted the gut microbiota of aquatic invertebrates in the Saint John River, New Brunswick, Canada. Sampling of macroinvertebrates from macrophytes, sediments and rocky habitats was conducted at several sites in October 2016 and in August 2017, and included Amphipoda, Trichoptera, Diptera, Ephemetoptera and Heterobranchia representing primary consumers through predators. Aquatic invertebrates were identified to the species level using the cytochrome c oxidase subunit I (*COI*) barcode. To assign taxonomic information to the bacterial sequences obtained from aquatic invertebrates, operational taxonomic units (OTUs) were generated using the V3-V4 hypervariable region of the *16S rRNA* genetic barcode at a 97% similarity threshold. The most abundant bacterial phyla across the gut microbiota of all aquatic invertebrates were Proteobacteria and Bacteroidetes, while the most abundant bacterial family was Enterobacteriaceae. It was found that the gut microbiota of aquatic invertebrates was related to invertebrate taxonomy – at the order and family levels – as well as their functional feeding group. For example, primary consumers had greater gut bacterial diversity, richness and evenness than predators. Additionally, water flow and substrate type also affected the gut microbiota. These findings will aid in establishing a baseline of natural variability and diversity upon which future hypotheses can be generated. This information is essential to determine before these techniques can be used to assess how or whether human activities affect aquatic organisms through their gut microbiome.

**611 Developmental estradiol exposure results in microbiota-dependent effects on locomotor activity in larval zebrafish**

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Both natural and synthetic endocrine disrupting chemicals are known widespread environmental contaminants. During early vertebrate development, estrogen receptor signaling plays an important role in proper brain and nervous system development and function. Because host-associated microbial communities play important roles in nervous system development and can biotransform xenobiotics, we hypothesized that microbial colonization status may influence the neurobehavioral toxicity and chemical toxicokinetics of exogenous 17beta-estradiol (E2), a classic estrogen receptor agonist. To test this, conventionally colonized zebrafish were developmentally exposed to E2 in a semi-static system. At 10 days post-fertilization (dpf), mortality was assessed. 16S rRNA gene sequencing was used to evaluate potential shifts in microbial community composition and predicted metagenomic function following exposure to five non-teratogenic E2 concentrations (0.34–3.5 µM). Locomotor activity was assessed in colonized and axenic (microbe-free) zebrafish exposed to 0.4 or 1.2 µM E2 using a standard light/dark behavioral assay and zebrafish tissue was collected and analyzed using targeted and non-targeted chemistry techniques. Developmental exposure to E2 did not alter microbial composition or predicted function. Locomotor hypoactivity was observed in the light epoch in E2-exposed larvae colonized with microbes only; axenic larvae exposed to E2 exhibited a normal behavioral phenotype in the light. Targeted chemistry analysis revealed that axenic zebrafish contained three times more E2 than colonized zebrafish. Combined, these results suggest that host-associated microbiota bioactivate E2, resulting in an altered behavioral phenotype. These data demonstrate for the first time that chemical-induced neurobehavioral toxicity is dependent on the presence of microbiota and suggest that current hazard identification strategies have the potential to misestimate toxicological risk if chemical-microbiota interactions are not considered. *This abstract does not necessarily reflect EPA policy.*

**612 Host-microbiome alterations of fatty acid metabolism in *Danio rerio* following dietary phthalate exposure**

*A. Buerger, University of Florida / Environmental and Global Health; C.J. Martyniuk, University of Florida / Physiological Sciences; J. Bisesi, University of Florida / Environmental and Global Health*

Dysbiosis of the gut microbiome is associated with various health outcomes, such as colorectal cancer, anxiety, and obesity. The unprecedented increase in obesity rates globally is suspected to be exacerbated by exposure to environmental chemical contaminants, referred to as obesogens. Chemical exposure can alter gut microbial diversity, resulting in changes in microbial function that ultimately impact host function. Phthalates, a class of plasticizers commonly found in food containers, medical devices, and personal care products, are ubiquitous environmental chemical contaminants and suspected obesogens. We hypothesize that exposure to a common phthalate, diethyl-hexyl phthalate (DEHP), in addition to overfeeding, disrupts microbial diversity in the gut microbiome, leading to increases in microbial short chain fatty acid production, and altered lipid uptake and metabolism in the host gut tissue, ultimately leading to exacerbated obesity. *Danio rerio* (zebrafish) were randomly assigned to one of three groups: 1) Control 2) Overfed or 3) Overfed + DEHP. The exposure lasted for 60 days, and body mass index (BMI) was calculated at three time points over the course of the study. On the final day, zebrafish were euthanized and gut and fecal matter were excised for sequencing. Zebrafish showed elevated BMI in Overfed and Overfed + DEHP groups. 16s microbial sequencing of fecal matter revealed significant changes in β diversity, increases in Bacteriodetes, and decreases in Fusobacteria and Tenericutes in Overfed + DEHP zebrafish compared to Control and Overfed fish. In Overfed + DEHP compared to Overfed alone, PICRUSt analysis revealed significant changes in carbohydrate, galactose, inositol phosphate, and taurine and hypotaurine metabolisms. Changes in microbial function were paralleled by changes in host functions related to lipid metabolism and gut function, as revealed by RNAseq analysis and qPCR. Taken together, these data indicate that following DEHP exposure, there is disruption of microbial diversity and alterations in the abundance of communities associated with fatty acid production, as well as alterations in expression of corresponding genes related to fatty acid uptake and anabolism in the enterocytes of the host gut. These data suggest a role for DEHP in exacerbation of obesity following overfeeding through microbial dysbiosis in humans and aquatic organisms alike, which should be considered when regulating their release into the environment.

## Environmental Assessments for Human and Veterinary Pharmaceuticals – Evolving Regulations

**613 What's new at the US Food and Drug Administration? Part 1: The Center for Veterinary Medicine**

*H. Zahner, E.M. Silberhorn, US Food & Drug Administration / Center for Veterinary Medicine; J. Laurenson, R. Bloom, US Food & Drug Administration / Center for Drug Evaluation and Research*

This presentation, which is the first of two US Food and Drug Administration (FDA) presentations, will discuss current regulations, guidance, policy, and approaches used at the FDA's Center for Veterinary Medicine (CVM) to prospectively evaluate the potential for environmental impact of new animal drugs. Under the National Environmental Policy Act (NEPA), FDA is responsible for determining whether the agency's actions (e.g., approval of a new drug) will result in potential environmental impacts. NEPA provides tools, such as environmental assessments (EA) and categorical exclusions from the requirement to prepare an EA, for evaluating the environmental safety of agency actions. In addition, CVM has published a Guidance for Industry (GFI) with recommendations on how to conduct an EA that was developed and harmonized with regulatory agencies in the European Union and Japan, and has since been adopted by others. This guidance recommends a tiered approach to testing that includes an evaluation of the fate, transport, and effects of animal drugs in the environment, and recommends using a traditional risk quotient approach when assessing risk. However, CVM's GFI is not applicable to the evaluation of some new animal drugs. For example, the current GFI does not provide agency recommendations on how to estimate potential environmental concentrations in soil or water, or on how to evaluate an animal drug that has an unusual route of exposure (e.g., dietary), is known to be toxic at very low concentrations (e.g., endocrine active compounds, parasiticides), those that bioaccumulate in organisms, or those that persist in the environment. Examples of novel approaches used by the animal drug industry and CVM to address current issues will be presented. In addition, CVM's current and future scientific perspectives regarding the EAs for animal drugs will be discussed. During this presentation, a comparison between FDA's Center for Drug Evaluation and Research (CDER) and CVM's approaches to environmental risk assessment will also be provided.

**614 What's new at the US Food and Drug Administration? Part 2: The Center for Drug Evaluation and Research**

*R. Bloom, J. Laurenson, US Food & Drug Administration / Center for Drug Evaluation and Research; H. Zahner, E.M. Silberhorn, US Food & Drug Administration / Center for Veterinary Medicine; C. Pinto, US Food & Drug Administration / OCSPP / OSCP*

This presentation, which is the second of two from FDA, will address environmental assessment (EA) review activities at the FDA Center for Drug Evaluation and Research (CDER). CDER evaluates the safety, efficacy and quality of drugs for human use. The EA review at CDER primarily evaluates exposures, toxicity, and effects to environmental organisms due to the introduction of human drugs into waters and soils from patient use and drug disposal. To date, many high production volume drugs treating common diseases have been evaluated and many are now marketed in generic forms. A review of more current applications shows the nature and number of new drug applications and, in particular, new molecular entities (NMEs), changing significantly over the last decade. Currently, increasing numbers of accelerated approvals, breakthrough therapies, orphan drugs, personalized medicine, and targeted drugs are being submitted. Many of these drugs are produced at low levels and would not ordinarily be expected to elicit a toxic response in the environment; however, additional evaluations may be necessary for drugs with high toxicity and bioaccumulation potential. In the past, such low volume production drugs usually received routine exclusions from the requirement to submit full EA packages. Recently, FDA published a GFI (*Environmental Assessment: Questions and Answers Regarding Drugs With Estrogenic, Androgenic, or Thyroid Activity*) to explain that applications that claim a categorical exclusion when expected effluent concentrations arewww.fda.gov/drugdisposal). The Team also works closely with the EPA Office of Water to help prioritize research and publications on pharmaceuticals introduced into the environment. CDER recently published two articles: one on the risk assessment of ethinyl estradiol and a second paper that examines risks associated with the disposal of human pharmaceuticals. The Drug Disposal website and publications will be briefly discussed.

**615 Future challenges and regulatory improvements in the assessment of environmental risks from veterinary medicines – a European Perspective**

*J. Weeks, ERA*

This talk will focus on the current and future developments in the environmental risk assessment of veterinary medicine products (VMPs) from a European regulatory perspective. There is a need to continuously evolve and react to changing requirements to perform ever more sophisticated, quantitative or robust environmental risk assessments for veterinary medicine products. The centralised registration of new veterinary medicines within the EU, is the responsibility of the European Medicines Agency (EMA). EMA frequently has need of and takes advice from specialist working parties aligned to the specific provision or modification of regulatory guidelines or procedures. One such group, the Environmental Risk Assessment Working Party focuses on the improvement of the guidance to industry and other stakeholders on environmental risk assessment alongside the provision of reflection documents that aid understanding or address specific areas for clarification in regulatory procedures. The overview will highlight some of these recent developments, in improved regulatory advice for current or future procedures. It will summarily cover issues around assessing and limiting veterinary medicines in groundwater, the use of higher tier testing of dung fauna, the use of higher tier plant testing, improvements in PBT assessments, future plans around developing improved guidance for the assessment of risks from aquaculture medicines and VMPs and AMR in the environment. The talk will compare developments in improving the guidance on the assessment of VMPs in the environment at the EU level and seek comparison to similar challenges in North America by regulators and industry alike.

**616 An investigation of sexual maturity status as a confounding factor in screening assays for the detection of androgens and anti-estrogens**

*K. Roush, M.K. Sellin Jeffries, Texas Christian University / Department of Biology*

Given the prevalence and effects of some human and veterinary pharmaceuticals, regulatory bodies have placed there an increased emphasis on identifying endocrine disrupting compounds. Some pharmaceuticals have been identified as reproductive endocrine disrupting compounds (REDCs) capable of altering sexual development, reproductive success and behavior. As such, the goal of this project was to determine whether sexual maturity status, as assessed via the prominence of secondary sexual characteristics, in female fathead minnows (*Pimephales promelas*) influences their response to REDCs. Specifically, we sought to determine whether less sexually mature females differed from more sexually mature females in their responses to 17β-trenbolone (TB, a common veterinary pharmaceutical) and fadrozole hydrochloride (FZ, a human pharmaceutical). Adult female fathead minnows were sorted by the prominence of secondary sexual characteristics, exposed to TB or FZ for 7 days and sampled for the analysis of estrogen-responsive gene expression. Results showed that fish with highly prominent secondary sexual characteristics responded differently to REDCs than fish with less prominent secondary sexual characteristics indicating that sexual maturity status influences REDC exposure outcome. For example, a decrease in hepatic vitellogenin expression was detected in the more sexually mature females but was not detected in the less sexually mature females after exposure to TB; therefore, had less sexually mature females alone been utilized, the defeminizing effects of TB would not have been identified. These results indicate that sexual maturity status should be taken into consideration in the implementation of screening assays as failure to utilize fish of the “appropriate” maturity status can skew test results. Ultimately, by refining existing screening assays to ensure adequate detection of pharmaceutical REDCs, environmental assessments of these compounds will improve.

**617 Developing Guidance to Support the Canadian Regulatory Proposal for the Environmental Assessment of Medicinal Ingredients in Human/Veterinary Drugs**

*J. Chateauvert, Health Canada / Environmental Impact Initiative Division*

Currently, manufacturers and importers of medicinal ingredients in drugs must notify under the *New Substances Notification Regulations* (NSNR) under the *Canadian Environmental Protection Act, 1999* (CEPA) in order for the Government of Canada to conduct an environmental risk assessment. A new proposed Canadian regulatory framework is being developed specifically for medicinal ingredients in human and veterinary drugs regulated by the *Food and Drugs Act* (F&DA) to assess risks to the environment and to human health resulting from environmental exposure. This new regulatory framework has been designed to align with the drug approval process stipulated by the F&DA and its regulations, and to harmonize with international jurisdictions. The creation of a new regulatory framework has provided Health Canada regulators with an opportunity to investigate and address concerns and gaps within the current NSNR system with respect to medicinal ingredients in drugs so that data requirements can be designed with cutting edge technologies in mind. Specifically, this presentation will describe how new draft guidance that will accompany the proposed regulatory changes under the F&DA will address requirements that are specific to human cell-based therapies, as well as substances that are replicating versus non-replicating, while striving to create a streamlined drug approval process that is comparable internationally. The purpose will be to obtain feedback from international counterparts and meeting attendees.

**618 Environmental Assessment of New Human Drugs in the US and EU – Lessons Learned**

*T.A. Verslycke, Gradient; T. Lunsman, Gradient Corporation / Ecological Sciences*

Market approval of new human drugs in two key markets, the US and the EU, requires an assessment of potential environmental risks associated with patient use. The framework for conducting environmental assessments (EAs) of new human drugs in these markets, however, is not harmonized. As a result, the scope, cost, complexity, and time needed to complete the EA requires careful and early planning by pharmaceutical companies to ensure compliance and a timely filing. New human drugs may also have specific properties (*e.g.*, anti-infective, endocrine active, ionizable, low solubility) that trigger the need for a more tailored EA. Currently, guidance by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for conducting tailored assessments is limited, leaving substantial room for interpretation and professional judgment. Using several case studies, this presentation will illustrate technical challenges and successful approaches for conducting tailored EAs for new human drugs in the US and the EU. We will also make recommendations to streamline and harmonize approaches to tailored EAs given current available guidance and state-of-the-science. For example, an approach will be presented for considering existing environmental exposures (*e.g.*, exposures resulting from veterinary and human drug uses and/or natural sources) when conducting an EA for endocrine active and anti-infective drugs.

**619 Environmental Assessment Compliance Under FDA’s New Guidance for Drugs with Estrogenic, Androgenic, or Thyroid Activity**

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AbbVie is developing a small molecule drug with effects on the endocrine system. The expected concentration (EIC) at the point of entry into the aquatic environment was below the 1 ppb criterion for categorical exclusion under FDA’s Guidance for Industry on the Environmental Assessment of Human Drug and Biologics Applications (July 1998). However, extraordinary circumstances applied given the hormonal activity of the molecule, which became subject to FDA’s Questions and Answers Regarding Drugs with Estrogenic, Androgenic, or Thyroid Activity Guidance for Industry (March 2016). AbbVie modeled the plasma concentration of the molecule in fish to inform the risk assessment, and found an effect ratio of 1200 (based on Huggett et al., Human Ecol. Risk Assess. 9:1789; 2003). This model assumes an effect ratio > 1000 might not warrant additional assessments in fish, but this model relies upon hydrophobicity to predict compound uptake and does not take into account factors such as hormonal activity. Environmental studies were requested by FDA. Consideration for the need for environmental studies included lack of examples of compounds with the same mechanism of action for “read across” purposes, and no data on environmental metabolism, fate, and transport of the AbbVie molecule. The environmental program was developed in a scientifically directed manner based on mechanism of action and in consultation with FDA. Studies and guidelines were as follows. Physicochemical properties (AbbVie internal methods), Partition Coefficient (OECD 107), Water Solubility (OECD 105), Activated Sludge Respiration Inhibition (OECD 209), Daphnia Magna Reproduction Test (OECD 211), Algal Growth Inhibition (OECD 201), Adsorption/Desorption on Soils & Sewage Sludge (OECD 106), Transformation in Aerobic and Anaerobic Aquatic Sediment Systems (OECD 308), Non-GLP Range Finding Trial for a Medaka Extended One Generation Reproduction Test (MEOGRT; OECD 240), Medaka Extended One Generation Reproduction Test (MEOGRT; OECD 240), Bioaccumulation in Fish (not triggered; OECD 305), Biodegradation in Activated Sludge (not needed; OECD 314B). Based on available data from these studies, the molecule is not expected to represent a risk to the aquatic environment. This presentation will review the process used for assessing environmental safety and the contributions and challenges associated with each study used to determine the fate and effects of AbbVie’s drug candidate.

**620 Improving the Interpretation of Results from the Fish Short Term Reproduction Test and the Medaka Extended One Generation Reproduction Test**

*H.O. Krueger, EAG Laboratories; S. Schneider, EAG Laboratories*

Higher tier endocrine tests like the FSTRA and MEOGRT are used to evaluate the potential for a chemical to cause endocrine disruption in fish. While variables such as fertility, fecundity, intersex and sex reversal have been identified as apical endpoints, other endpoints are used to evaluate mechanistic outcomes. Mechanistic endpoints generally include secondary sex characteristics, vitellogenin, gonad pathology and plasma steroids. While guidance documents provide performance criteria and statistical methods for the analysis of these variables, there is little guidance on how to integrate all the variables into an overall strength of evidence assessment to determine whether a chemical has endocrine activity, or if it should be considered an endocrine disruptor (i.e., has adverse health consequences for individuals or populations). Not only is it important to evaluate statistically significant trends, but equally important is to determine whether the magnitude of the responses are statistically significant and biologically relevant relative to controls. We argue that the pattern of the response is equally important to the response of an individual variable. It is also important that variables are weighted in considering their importance in an overall pattern of responses. Jensen and Ankley (2014) provide guidance on breaking out results into overt toxicity, apical endpoints, and mechanistic endpoints for FSTRA studies, while Borgert et al (2011) have used weight of evidence methodology to evaluate endocrine activity across suites of studies. We have built on these methodologies to compare responses of known endocrine disruptors to the test chemical in order to identify signature patterns of responses of known ER-agonists, AR-agonists, or AR-antagonists for fish FSTRA and MEOGRT. Such an approach helps prevent using spurious results to draw erroneous conclusions in evaluating endocrine disruption in fish.

## California Oil Spills: Impacts on Habitat and Wildlife

**621 Overview of impacts to natural resources from the Refugio Beach Oil Spill, Santa Barbara, California**

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Following the Refugio Beach Oil Spill, May 19, 2015, along the Gaviota Coast, state and federal trustee agencies pursued a cooperative natural resourcedamage assessment (NRDA) with the responsible party, Plains, LLC. The goal of the assessment was toquantify injuries to wildlife,habitat, and lost uses of those resources,and then to determine how to best restore the resources and compensate for the losses. In the course of the NRDA, the trustees conducted over 50 studies. This presentation provides an overview of the injuries assessed, including impacts to birds, mammals, fish, shoreline and subtidal habitats, as well as human recreation. The ultimate goal of the trustees is to restore and compensate for the injured resources. Following public outreach efforts, restoration projects will be proposed for implementation along the Southern California coastline.

**622 Applying Multiple Forensic Tools to Distinguish Spilled Pipeline Oil from Natural Seeps Following the Refugio Beach Oil Spill**

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During the 2015 Refugio Beach Oil Spill in Santa Barbara County, CA, an estimated 190,000 liters of Monterey formation crude oil spilled from an onshore pipeline and flowed into the surf zone. Once in the ocean the oil was transported by currents, waves, wind, and other natural processes to nearshore, offshore, and subtidal zones. Some of the oil stranded on the shore and was found more than 200 kilometers from the spill site; some oil mixed in the nearshore and sank. Oil that was not recovered during response operations was gradually altered by various weathering processes. Numerous productive natural seeps release oil and natural gas from the seafloor in the Santa Barbara Channel region; similar seeps occur in nearshore zones throughout the Southern California Bight. Since the crude oil being transported in the Line 901 pipeline at the time of the spill was derived from offshore oil extraction platforms in the Santa Barbara Channel, forensically characterizing and identifying the provenance of oil found in the environment after the spill required the use of several forensic techniques and tools to distinguish spilled oil from natural seep oils. Natural seep oils have distinguishable characteristics and have been studied extensively by the U.S. Geological Survey and others. Despite originating from common subsea geological formations, careful analysis and interpretation using multiple lines of evidence provided the means to fingerprint samples of stranded oil, floating oil, and oil in other matrices, and reveal the transport and fate of oil spilled from the Line 901 pipeline.

**623 Polycyclic aromatic hydrocarbon uptake and forensic results in three beach invertebrate tissues and porewater – Refugio State Beach Oil Spill, 2015**

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Sediment porewater and three sandy beach invertebrate species from Santa Barbara County beaches affected by the 2015 Refugio Beach oil spill were analyzed for polycyclic aromatic hydrocarbons (PAHs). A distinguishable PAH pattern was identified in tissue and porewater samples collected at several locations and multiple time points post spill. Significant correlations between porewater and tissue residues were identified. The most elevated PAH concentrations were observed in beach hoppers (*Megalorchestia spp.*), and the results showed elevated PAH concentrations persisted up to 4 months post spill in some locations, a longer duration than was observed in the sand crabs (*Emerita analoga*) or polycheates (*Thoracophelia spp.*). Sand crabs took up PAHs rapidly after the spill, within hours to days, but also appeared to have metabolized them rapidly. Forensic chemistry matches were identified between the source oil, taken near the ruptured oil pipeline, and multiple tissue types, as well as porewater samples. Beach hoppers and porewater served as useful monitoring media for documenting decreasing trends of PAHs in sandy beaches following this spill event.

**624 Population responses of sandy beach talitrid amphipods to the Refugio Beach Oil Spill, Santa Barbara County, 2015**

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Open coast sandy beaches in California harbor a rich intertidal community of macroinvertebrates, including talitrid amphipods. The talitrid amphipods or beach hoppers *(Megalorchestia spp.*) are important intertidal animals of open coast sandy beaches on the California coast. These nocturnal animals burrow in the damp sand of the upper intertidal zone in the day and emerge at night to feed on stranded macroalgae, such as giant kelp and feather boa kelp. They are dominant consumers of drift macrophytes or wrack on beaches in California and their feeding activities contribute to detrital processing and nutrient cycling. To evaluate possible injury to beach ecosystems resulting from the 2015 Refugio Beach oil spill in Santa Barbara County, we surveyed populations of beach hoppers on a number of affected beaches. Surveys were conducted at a range of beach locations and at multiple time points following the spill. Significant declines in beach hopper population abundance and biomass were observed at affected beaches in June 2015 when compared to available baseline data. Populations of beach hoppers continued to decline through summer 2015, likely due to continued oil spill cleanup activities. Results of surveys in June 2016 and 2017 suggest recovery of talitrid populations was protracted at beaches affected by the oil spill.

### 625 Development of a Polycyclic Aromatic Hydrocarbon Bioassay Using Sand Crabs (Emerita analoga) following the Refugio Beach Oil Spill, Santa Barbara, CA

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When developing toxicity bioassays for the purpose of oil spill natural resource damage assessment (NRDA), it is important to take into consideration key properties of polycyclic aromatic hydrocarbon (PAH) compounds, and to utilize appropriate organisms whenever possible. Therefore, an exposure scenario related to an oil spill event should take into consideration solubility, declining concentrations, and temperature. These parameters are important because each effects how PAHs interact once introduced into the marine environment. Similarly, care must be taken to select the appropriate organism(s) for use in toxicity bioassays. These organisms should be indicators of ecosystem health, available for collection, indigenous (if possible), and of a known age class. Additionally, these organisms should exist throughout a broad geographic range and should be easy to collect, sort by size, and maintain in a laboratory. For these reasons, *Emerita analoga* was selected for this bioassay.

### 626 Surf Zone Water and Beach Porewater Chemistry Following the Refugio Beach Oil Spill Indicate Effects to Fish and Invertebrate Early Life Stages

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During the 2015 Refugio Beach Oil Spill in Santa Barbara County, CA, Monterey formation crude oil spilled from an onshore pipeline and an estimated 190,000 liters entered the surf zone, where it actively mixed with seawater and was transported along the shoreline and offshore. Immediately following the spill, dead adult fish and invertebrates were observed in the spill affected area. Early life stages of many marine aquatic animals were present at the time of the oil spill that have been shown in previous studies to be sensitive to adverse effects from oil exposure. To evaluate exposure, water samples were collected from several locations between Gaviota State Beach and Carpenteria State Beach from May to September 2015. Elevated concentrations of polycyclic aromatic hydrocarbons (PAHs) and total petroleum hydrocarbons (TPH) were measured in the surf zone water and beach porewater samples collected near the release, declining over space and time. To investigate toxicity to early life stages, sand crab megalopae (*Emerita analoga*) and larval inland silverside (*Menidia beryllina*) 6-7 day static renewal bioassays were conducted. A high energy water accommodated fraction (HEWAF) mixture of the source oil and seawater was prepared and used in the laboratory bioassay studies to simulate surf zone contaminant exposure conditions. The oil-water proportions in the HEWAF preparations were designed to generate PAH exposure conditions that bracketed the measured concentrations in field collected water samples at the time of the spill. PAH composition and concentrations of the HEWAF were chemically analyzed to confirm that the bioassay exposures generally mirrored those shown to have occurred in the nearshore environment after the spill, based on chemical analysis of field collected water samples. Bioassay results indicated PAHs and TPH concentrations in surf zone water and porewater were lethal to early life stages of fish and invertebrates.

### 627 Effects of oiling on the seagrass, Phyllospadix torreyi, as a result of the Refugio Beach Oil Spill, Santa Barbara County, 2015

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On May 19, 2015, crude oil spilled from a pipeline rupture on land and entered the surf zone and spread to nearshore habitats in the area of Refugio State Beach, Santa Barbara County, California. Oil from the spill entered sensitive marine habitats, in particular beds of the surfgrass, *Phyllospadix torreyi*, a marine angiosperm that grows abundantly in the low-intertidal/shallow subtidal. Surfgrass is one of the most productive marine primary producers, and provides important shelter, foraging areas, and nursery habitat for fishes and invertebrates, such as the commercially important California spiny lobster. Because of its importance, researchers studied the effects of the oiling on this species of surfgrass. Researchers observed a significant change in the condition of the surfgrass leaves beginning two months after the spill. Notably, the surfgrass leaves turned in color from a typical emerald green to yellow, brown, and black. In addition, the leaves were brittle and broke apart easily when pulled by hand. Quantitative surveys over the next year further assessed the health and condition of surfgrass and associated algae that had also became discolored. Quadrats, transects, and sampling by paddle board, snorkel, and SCUBA surveys were utilized to document the degree of injury to intertidal and shallow subtidal surfgrass beds. Effects were measured at all sites where there was heavy oiling.

### 628 Impact of the Refugio Beach Oil Spill on Two Surf Zone Fish: Surfperch (*Embiotocidae*) and California Grunion (*Leuresthes tenuis*)

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During the May 2015 Refugio Beach Oil Spill, 190,000 liters of crude oil flowed into the surf zone. Fish in the nearshore environment were exposed to the oil by direct contact, and via consumption of contaminated prey. As part of the subsequent Natural Resource Damage Assessment, studies were conducted to examine the impacts of oil exposure on two groups of surf zone fish: surfperch (Embiotocidae) and California grunion (*Leuresthes tenuis).* A quantitative assessment of exposure was conducted by measuring levels of polycyclic aromatic hydrocarbon (PAH) metabolites in surfperch bile. Five days after the spill, surfperch were collected at Refugio State Beach, a heavily oiled area, Gaviota State Beach, a lesser oiled area, and Campus Point Beach, an area with potential seep oil exposure. Surfperch had significantly elevated levels of biliary PAH metabolites at Refugio State Beach, compared to the other locations. Effects on early life stages of the California grunion were evaluated due to their unique life history which includes burying fertilized eggs on beaches where they incubate until being washed out to sea two weeks later. Grunion beach spawning events were observed in June 2015 and egg clutches were collected 5-7 days later at two beaches in the spill affected area and at two relatively unoiled beaches. Spawning observations and egg clutch collections were repeated one year later. Field collected eggs were incubated in the laboratory, then triggered to hatch by seawater agitation. Hatching behavior and survival were monitored. In general, eggs from grunion clutches that were collected from beaches that were exposed to oil had lower hatch rates, compared to clutches that were collected from beaches that were not exposed to oil. These studies demonstrated that surf zone fish were exposed to oil and indicated that adverse effects to early life stages of fish occurred.

# Environmental Risk Assessment of UV Filters in Freshwater and Marine Environments

**629 Environmental Risk Assessment of UV Filters in Freshwater and Marine Environments: State of Science and Challenges**

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UV filters are ingredients in personal care products that protect consumers against the adverse effects of UV radiation. UV filters are also used as photoprotective agents in other products, such as plastic and apparel items. Organic UV filters have diverse chemistries and moieties. Inorganic UV filters are metallic and can be nanoscale or non-nanoscale. UV filters such as benzophenones share chemistries with naturally occurring compounds. For example, oxybenzone is a natural compound produced by higher plants. It is crucial to appreciate these various chemistries and sources when assessing environmental fate and effects of UV filters. Environmental exposure to UV filters occurs *via* point and diffuse pathways. Point exposure occurs when UV filters wash-off bathers. This includes exposure to coastal sediment when, for example, aerosolized UV filters are applied to people on a beach. Diffuse exposure occurs when UV filters are washed-off down the drain and enter the waste water system. UV filters have been detected at low ng/L to µg/L levels; mainly in the marine environment. However, more monitoring studies are needed before a clear picture of exposure can be painted; the first step in an environmental risk assessment. When UV filters enter the aquatic or marine environment they are subject to various fate and transport mechanisms. Very little is known about these mechanisms, yet such data are essential to understand the concentration and timing of potential exposure of organisms to UV filters and/or their metabolites/degradation products. Further fate and transport research is therefore required to establish partitioning of UV filters between water and organic phases, biotic and abiotic degradation pathways and bioaccumulation of these materials. In addition to the limited amount of data regarding the concentration of UV filters in the environment, very few ecotoxicological data are available to assess whether UV filters are hazardous at environmentally relevant concentrations. Although some laboratory ecotoxicological studies have been conducted for a handful of UV filters, many questions remain over the extrapolation of these studies to the environment. Overall, huge data gaps exist for UV filters with regard to exposure and toxicological hazards, including endocrine activity. More research is therefore required to fill these data gaps and establish the risk these materials pose to organisms residing in the marine and aquatic environment.

**630 Targeted and non-targeted analysis of coastal contaminants in coral reefs of Oahu, Hawaii**

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Coastal water samples and sediments were collected in shallow waters near public beaches, and waters beyond the barrier reef using a small vessel along the coast of Oahu, Hawaii. The objective of this study was to investigate the presence of known anthropogenic contaminants, for example polycyclic aromatic hydrocarbons (PAHs), as well as screening for suspected contaminants using a non-targeted ultrahigh resolution Fourier transform ion cyclotron resonance mass spect5rometric (FT-ICR MS) technique. Results showed that of the 49 PAHs examined, some were present at all beach locations, although the composition of the PAHs differed slightly. These findings are of concern as PAHs have been shown to concentrate and cause impacts in corals. The non-targeted approach revealed a large diversity of aliphatic and aromatic sulfonic surfactants, their co- and by-products, which highlights that coastal waters of Hawaii, and in particular near-shore coral reefs, are exposed to a multitude of contaminant stressors. The linear alkyl benzene sulfonates (LAS) are of particular concern, because they showed in previous studies very high toxicity to corals. These findings suggest corals off Oahu, Hawaii are exposed to a variety of potentially toxic compounds, adding to the stresses already imposed by increased ocean temperature.

**631 Concentration of the UV-filters Oxybenzone and Octinoxate in Coral Tissue (*Porites* spp.), Surface Water and Sediment Samples from Oahu, Hawaii**

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UV-filters contained in sunscreen products, notably oxybenzene (BP-3) and octinoxate (OCX), have been the focus of recent legislation in Hawaii, USA, due to concerns regarding their potential toxicity to coral reefs. However, limited information is available on the environmental concentrations of these chemicals in seawater surrounding Hawaiian coral reefs, and no data have been reported for the environmental distribution of these chemicals in coral tissue and sediment from Hawaii. To address these knowledge gaps in October 2017 we collected surface seawater, coral tissue (*Porites* spp.) and sediment samples from 19 sites in Oahu, Hawaii that differed in potential inputs of sunscreen-derived UV-filters. Nearshore and offshore sampling sites were chosen at Waikiki Beach (popular tourist destination), Kaneohe Bay (tourist and mixed use site), and Ka'a'awa (chosen as a 'reference' location). An additional study included sampling seawater and sediment at a variety of beach parks around Oahu. The UV-filters, BP-3 and OCX were measured using LC-ESI-MS/MS techniques. BP-3 was detected in seawater at all 19 sites, but the aqueous concentrations were less than 10 ng $L^{-1}$ at 12 out of the 19 sites. At Waikiki Beach, BP-3 concentrations were as high as 136 ng $L^{-1}$. Similarly, BP-3 was detected in all coral tissue samples at concentrations of 17-265 ng $g^{-1}$ (dry weight; dw). However, BP-3 was only measurable in sediment samples at 7 out of 19 sites at < 1 ng $g^{-1}$ (dw). No quantifiable levels of OCX were recorded for the seawater or coral samples, although OCX was quantified in sediment samples from Kaneohe at concentrations less than 13 ng $g^{-1}$ (dw). This study provides insight as to the environmentally relevant concentrations in seawater and sediment in Hawaiian coral reef locations. In addition, this study is the first to report concentrations of UV-filters in corals from the USA. These data provide an important baseline for future risk assessments of the potential toxicological effects of UV-filters on coral reefs and other marine organisms in Oahu, Hawaii.

**632 Anaerobic wastewater and marine microbial communities demethylate pharmaceuticals and the UV filter oxybenzone with metabolite accumulation**

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Pharmaceuticals and personal care products (PPCPs) can be metabolized by microbes, releasing not only the intact PPCPs but also their metabolites to the environment. The multitude of PPCPs makes studying each microbial metabolism an arduous task. We propose that predicting microbial metabolism of PPCPs during wastewater treatment is best understood in the context of shared chemical structure. We recently described a methanogenic enrichment culture from anaerobic wastewater sludge that readily demethylates the common pharmaceutical naproxen (Aleve) to 6-O-desmethylnaproxen, with no loss of the metabolite observed. In this culture, methane was generated at stoichiometric amounts to naproxen demethylation, indicating that the methyl group removed from naproxen stimulates a complex microbial community. The natural methoxy aromatic compounds syringic acid and vanillic acid were also readily demethylated, and this shared metabolism prompted an examination of other methoxy aromatic xenobiotic compounds. In the present study we challenged the methanogenic wastewater culture with the methoxy aromatic UV filter oxybenzone and observed demethylation to benzophenone-1, which itself is an estrogenic contaminant. In addition, the wastewater culture transformed the pharmaceutical guaifenesin (Mucinex) to a novel demethlated metabolite whose environmental and health impacts are yet unknown. A second culture from sulfidic marine sediment was established to simulate anoxic environmental sediment receiving pharmaceutical contamination. Again, the natural and PPCP methoxy aromatic compounds were readily demethylated with accumulation of the metabolites. The demethylation of oxybenzone to the contaminant benzophenone by both cultures emphasizes the importance of methoxy aromatic demethylation in evaluating PPCP risk. This study suggests that methoxy aromatic PPCPs are readily transformed to demethylated metabolites both during anaerobic wastewater treatment and in the environment, and their associated metabolites may accumulate in anoxic environments.

**633 Direct dosing of an oxybenzone-based sunscreen formulation causes negative effects within the physiology and microbiome of two coral species**

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Reef-building corals are a vital component of biological diversity within the oceans and provide an economic engine for many coastal communities. Yet corals are experiencing high rates of mortality from a broad range of global and local stressors. Personal-care products, such as oxybenzone-based sunscreen may be one of them. We exposed colonies of *Montipora capricornis* and *Acropora cervicornis* to a range of oxybenzone concentrations (3 mg/L, 0.3 mg/L, 0.04 mg/L, 0.007 mg/L BP3, 0 mg/L oxybenzone) by dosing aquaria water with a particular sunscreen formulation every 48 hours for 17 days. To quantify the physiological impacts from exposure, we conducted daily visual assessments of the coral health, measured photochemical efficiency of photosystem II within the algal symbionts of the corals, the growth rates of *M. capricornis*, and the mortality rate of both species through time. We also measured the concentration of *Vibrio* sp. bacteria within the water column and within the mucus of *A. cervicornis* using selective media for this bacterial group. *Vibrio* concentrations were measured prior to dosing, 24 hours after dosing, and at the end of the experiment. After 17 days, *A. cervicornis* in the 3.0 mg/L and the 0.3 mg/L oxybenzone treatments and all of the *M. capricornis* exposed to sunscreen were either visually bleached or pale. Growth rates of *M. carpricornis* were also reduced at oxybenzone concentrations of 3 mg/L and 0.3 mg/L. All of the corals in the 3 mg/L oxybenzone treatment suffered 100% mortality within nine days. There was also a reduced maximum electron transport rate within the 3 mg/L oxybenzone treated *M. capricornis* and *A. cervicornis* and a reduced maximum effective quantum yield for *M. capricornis* in the 3 mg/L oxybenzone treatment. Prior to dosing, the seawater and the coral samples both contained less than one colony forming unit (CFU) per 150 ul of sample. After dosing, the number of *Vibrio* bacteria within both sample types significantly increased. However, concentrations reduced back to pre-treatment levels for both seawater and coral samples by the end of the experiment. In general, *M. capricornis* was more sensitive to the effects of the oxybenzone-based sunscreen compared with *A. cervicornis,* although both species were negatively affected by the exposure. Our results show that some oxybenzone-based sunscreen formulations can alter the host microbiome, reduce coral health and growth rate, and increase mortality rates of two coral species.

**634 UV filters contribution to coral bleaching? Laboratory chronic toxicity testing and concentration monitoring in the lagoon of the Moorea island**

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Some studies have reported that certain UV filters (mostly 4-methylbenzylidene-camphor, benzophenones and octylmethoxycinnamate) contained in sunscreen lotions and washed off by swimmers could trigger coral bleaching. Suspicion has been extended to all organic UV filters present in sunscreens products. The present study will report on the laboratory coral chronic toxicity assessment of some UV filters used in sunscreens (Octocrylene, Avobenzone, Terephtalylidene-dicamphor sulfonic acid, Silatrizole, Ethylhexyltriazone and the mineral UV filter Zinc oxide). Herbicides (Monuron and Diuron) and Tributyltin were used as reference toxic compounds. Cultured coral nubbins of the species *Stylophora pistillata* were exposed for 5 weeks to low UV filters concentrations (from 10 µg/L to 5 mg/L), but still higher than those reported in natural sea waters. Nubbins were exposed in 15-liters aquariums with weekly water renewal. UV filters concentrations were monitored all along the exposure period with automated solid phase extraction with UPLC-UV detection. Bleaching was visually observed and Photosystem II inhibition of the symbiotic zooxanthella was measured with Pulse Amplitude Modulated fluorometry, 5 days a week for 5 weeks. To enlarge the scope of these UV Filters impact assessment on aquatic biodiversity, additional ecotoxicity testing was performed with complementary soft and marine planktonic organisms such as micro-algae (chlorophytes and diatoms), daphnids crustaceans, fish embryos, marine bacteria and sea urchin larvae. Meanwhile, passive samplers were used to quantify aquatic UV filters concentrations at different sites of the lagoon of the Morea Island, in the French Polynesia. These chronic toxicity and field monitoring data provide new insights into the assessment of UV filters impact on coral reefs.

**635 Dibenzalacetone and its Derivatives: Sunscreen Agent (An Active Ingridient in Suncreens)**

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Sun energy is responsible for the growth of vegetation on which all life depends, directly or indirectly. Too much exposure to it can lead to adverse environmental health effect such as skin rashes, skin cancer, eye cataract, sun burn to mention a few. Sun protection is a significant public and environmental health issue, and sunscreens play an important role in the management of this risk. Sunscreens are agents that are designed to suppress the effect of sun radiation on the skin. An important component of sunscreen formulation is dibenzalacetone and its derivatives because of their ability to absorb and reflect UV radiation without adverse effect. Sunscreens utilize dibenzalacetone due to the fact that it absorbs UV rays, preventing them from absorbing into the skin itself. Other chemicals within sunscreen include those that actually reflect UV rays, pairing up with absorbers like dibenzalacetone to prevent as much skin absorption as possible. Dibenzalacetone is a highly potent chemical that absorbs UV rays, making it the ideal ingredient for sunscreens. In fact, dibenzalacetone is a leading ingredient in most commercial sun protection products, known for its highly stable and predictable properties. The chemical is also used because its stability allows it to be paired with other compounds commonly found in sunscreen, making SPF (Sun Protection Factor) levels easy to adjust. In this research study, some analogues of dibenzalacetone viz: 3,3'-dichlorodibenzalacetone, 4,4'-dichlorodibenzalacetone, 4,4'-diflorodibenzalacetone, 3,3'-dihydroxy-4,4'-methoxydibenzalacetone were prepared. The compounds were synthesized by adol condensation using 2 moles of the respective substituted benzaldehyde and 1 mole of acetone in the presence of sodium hydroxide. The compounds were characterized using melting point, FR-IR, UV-visible spectroscopy. The infra-red spectra of the compounds confirm the functional groups expected. The presence of vibrational peaks in the range 1442 – 1650 $cm^{-1}$ are evidence of C=C. The stretching frequency observed in the range 1642 – 1650 $cm^{-1}$ are attributed to C=O in the compound. The small peaks around 1572 – 1888 $cm^{-1}$ shows band position of aromatic ring. The wavelength of maximum absorption for the compound ranged from 278 – 324 nm while the molar extinction coefficient ranged from 5080 – 19520 nm. The spectroscopy data for the compounds were correlated with substituent constant using a form of Hammett equation.

**636 Balancing Human Health Benefits of UV Filters Versus Potential Environmental Risks**

*J. Sirois, CHPA / Regulatory & Scientific Affairs*

Regular sunscreen use is a proven method of reducing the skin damaging effects of UV radiation and can prevent melanoma and other non-melanoma skin cancers. Professional and medical organizations including the American Cancer Society, the American Academy of Dermatology and the Centers for Disease Control and Prevention recommend the use of a broad-spectrum sunscreen, or one that protects against both UVA and UVB radiation. Dermatologists agree that the best sunscreen is the one that is used on a regular basis. Factors contributing to effective use of sunscreen include cost, skin feel, and product performance. In the US, a limited number of sunscreen active ingredients are available which provide broad spectrum protection. Consumer preference is an important determinant of use and reducing the number of available sunscreens is very likely to result in reduced use, a situation that could lead to increased skin damage and skin cancer diagnoses. To date, a very limited number of studies have associated particular sunscreen active ingredients with harm to coral reef populations. These studies have been conducted under artificial conditions in a lab setting, have a number of inherent methodological limitations and have yet to be replicated under real-world conditions. A much larger body of work exists pointing to global climate change as the dominant contributor to coral decline worldwide. Many of these studies demonstrate the occurrence of coral reef bleaching irrespective of whether an area experiences high levels of human activity such as swimming, strongly suggesting sunscreens are not primarily responsible for the bleaching events. When translating science into governmental policy, decisions should be based on sound data. When viewed in its entirety, the currently available evidence on the causes of coral reef bleaching and mortality, both in HI and the world over, overwhelmingly demonstrates that climate change, ocean acidification and runoff are primarily responsible for coral bleaching. Banning individual sunscreen ingredients is unlikely to result in a significant benefit to coral reef populations while conversely creating the potential for significant public health implications associated with enhanced exposure to UV radiation. Sunscreens marketed as being “reef safe” apparently base this claim on the absence of a particular ingredient(s) and there is currently no regulatory authority or governmental body that recognizes this claim.

## Assessment and Monitoring of Risks to Aquatic Environments from Short-Term (Episodic) Contaminant Exposures

**637 An overview of the nature of episodic water pollution and implications for pollutant averaging periods to be protective of aquatic life**

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Contaminant concentrations and factors that modify their potential toxicity are time-varying in mid-size rivers and streams, depending on inter-annual, seasonal, or daily cycles, as well as stormwater-driven pulses. Short-term (< 1 day) contaminant exposures are difficult to monitor and can affect water quality. Aquatic life criteria in the USA intend to address the episodic nature of water pollution through a two-number criteria paradigm, in which different averaging periods are intended to account for time-varying exposures of different contaminant magnitudes. Using the Mancini kinetic model, toxic averaging times were calculated for a variety of acute tests, with mortality occurring in exposure times ranging from < 2 to >96 hours. However, with short-term acute exposures, delayed mortality can also be an important issue and the inability to account for delayed mortality following short-term exposures was a major limitation of most datasets examined. Averaging periods for acute criteria need to consider the commonness of episodic pollution events. The studies reviewed show short-term exposure to contaminants in stormwater runoff can be toxic to freshwater organisms, with several studies demonstrating episodic exposures of ≤3 hours could produce pronounced acute toxicity.

**638 Understanding episodic exposures: Acute and latent effects of zinc on Ceriodaphnia dubia and Oncorhynchus mykiss**

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Water pollution sources are often episodic, which results in time-varying concentrations in receiving waters. Runoff from built up or disturbed areas such as urban areas, highways, agriculture, or industrial, agricultural and mining operations can result in episodic pollution events. One of the challenges in assessing the effects of these pulsed exposures is that thresholds are often derived from acute 48- or 96-hour bioassays. A review of literature, from which time-to-effects in short-term (≤24 hour) bioassays or following pulse exposures could be sufficiently determined, shows that short-term exposures to a number of contaminants can be lethal to a variety of aquatic organisms. Additionally, several studies have reported that mortalities continue to accrue following short-term exposures to a metal, suggesting that evaluations of time-to-effects for metals may fail to consider delayed mortality. A series of experiments using the cladoceran *Ceriodaphnia dubia* and juvenile rainbow trout *Oncorhynchus mykiss* were conducted to evaluate the toxicity of zinc in up to 48-hour exposures with a cladoceran and in up to 96-hour exposures with rainbow trout, and to evaluate the latent mortality following the exposure to five or six concentrations of zinc. Test designs used a non-renewal test with the cladoceran for 1, 3, 8, 24, and 48-hour exposures and a static-renewal test with the trout for 1, 3, 8, 24, and 96-hour exposures (water renewal at 48 hours). Surviving organisms after the exposures were placed in the control water and latent mortality was monitored through the 48-hour experiment with the cladoceran and the 96-hour experiment with the trout. For the conventional acute exposures (48 hours with the cladoceran, 96 hours with the trout), surviving organisms after the exposures were placed in the control water and fed for 48 hours to determine whether latent mortality occurred. Results of these studies will be used to determine whether existing data are sufficient to demonstrate potential harm from brief, severe pulse exposures to toxicants, such as those that may occur during urban stormwater events. These results also give support to include provisions in water quality criteria framework to protect against brief or episodic conditions.

**639 A methodology to predict toxicity potential of an urban stormwater discharge**

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Urbanized areas are sources of stormwater runoff that may contain numerous potential contaminants and have varied intensity and duration. Therefore, biological effects of urban stormwater discharges may not be adequately addressed using traditional toxicological approaches, which are based on prolonged, constant exposures to single chemicals. This presentation uses aspects of an approach developed in the Pellston workshop: *Simplifying aquatic assessments of chemical mixtures in urban environments* to evaluate toxicity potential of Chollas Creek, a heavily urbanized drainage in San Diego, CA that is stormwater-dependent. Rainfall data from 10 separate wet weather events between 2009 and 2014 were related with acute toxicity tests and Event Mean Concentrations at two sites for over 60 chemicals including dissolved metals, PAHs, pyrethroid, chlorinated, and organophosphorus pesticides, and PCBs. Each chemical concentration was converted to toxic units (TUs) based on *Ceriodaphnia* acute EC50s and chemicals were ranked in terms of their contribution to the overall sum of TUs (concentration additivity) for each wet weather event. In decreasing order, bifenthrin, zinc, copper, malathion, and benzo(a)anthracene contributed between 60 and 75% of the total TUs. All pyrethroids, copper, zinc, malathion, and PAHs contributed between 88 and 96% of the total TUs in each stormwater sample. Total TUs based on 6-12 hour exposure (the duration of stormwater events at this site) was

### 640 Improving the assessment of potential impacts from intermittent, short-term, effluent discharges to aquatic environments

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Determining the risk posed by intermittent, fluctuating and pulsed contaminant discharges to aquatic environments is challenging. Water quality criteria (WQC) and whole effluent toxicity (WET) test methods are the most frequently utilized assessment tools used to predict or assess toxicity of effluents following mixing with the receiving water. However, both WQC and WET approaches were developed to assess continuous rather than intermittent contaminant exposures. Conservative assessment outcomes are desirable owing to the often complex and fluctuating composition of effluents, the nature of receiving environments, and varying organism/receptor responses. However, highly over-conservative assessments of short-term discharges may result in unnecessarily high costs for treatment or remedial actions. In this presentation we discuss toxicity results from short term (e.g. 1 to 48 h pulses) and chronic (e.g. days to weeks, depending on species lifecycle) exposures to individual contaminants and complex contaminant mixtures across a range of freshwater and marine microalgae and invertebrates (amphipods, copepods). For the range of species and test conditions (contaminants and pulse durations), observed toxicity was generally proportional to the time-averaged concentration (TAC) of the contaminant (or % effluent), i.e. inversely varying pulse concentration and duration resulted in a similar level of effects. The medium-term pursuit of this research is to determine the conditions under which (i) WQC may be relaxed by a factor proportional to the TAC, and/or (ii) WET methods may be applied with effluent exposure durations that better match the receiving environment, and achieve protective but not overly-conservative outcomes. Due to the limited range of receptor endpoints (species, test outcomes) and effluent contaminant mixtures assessed to date, there remains uncertainties that impede recommendations for modified risk assessment approaches. Knowledge gaps include contaminant mixtures, organism life-stage sensitivity, and the timing of exposure (day or night). Ultimately this research will facilitate more reliable assessment and management of intermittent discharges, such as stormwater runoff and those from industrial facilities.

### 641 Predicting the toxicity of episodic (plused) exposures of copper and zinc

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Metal toxicity to aquatic organisms has traditionally been studied by exposing organisms to a continuous concentrations for the duration of the toxicity test. This experimental design may be appropriate for discharges that are consistent for long periods of time (such as wastewater treatment plants). However, for discharges that are intermittent or time-variable continuous exposures may not be a realistic description of environmental conditions. Unfortunately, tools for understanding and predicting the effects of time-variable discharges have not been available to regulators and risk assessors. In this work we demonstrate that toxicity from time-variable exposures is reduced, compared to what would be expected from comparable continuous exposure conditions. For both copper and zinc, toxicity to sensitive aquatic organisms is shown to directly relate to exposure duration. The results from these tests show that comparable continuous exposures may overestimate toxicity by 10 or 100 fold. We also have developed a modeling framework for understanding and predicting toxicity from episodic exposures called the Biotic Ligand Episodic Exposure Model (BLEEM). BLEEM is an extension of the Biotic Ligand Model (BLM) that includes a time-variable damage and repair. Application of BLEEM to episodic copper exposures results in very good predictions of metal toxicity in time-variable and episodic exposures. BLEEM can be used as a regulatory and risk assessment tool for improving our understanding of the impacts of episodic metal exposures on aquatic life.

### 642 Preliminary Evaluation of Improved Toxicity Testing Methods for Episodic Discharges

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Episodic discharges such as stormwater require environmentally-relevant, scientifically-defensible, and conservative toxicity test designs to assess potential for receiving water impacts. Permittees in highly industrialized areas are increasingly required to conduct 96-h (or longer) static toxicity tests on stormwater collected at end-of-pipe associated with events that are generally less than 24-h. Our team is currently evaluating alternative toxicity test designs for episodic exposures that would require minimal modification to standard methods, yet increase realism towards protection of beneficial uses from episodic discharges. Preliminary results associated with this work include those from chronic toxicity tests with purple sea urchin (*Strongylocentrotus purpuratus*) embryos and acute toxicity tests with the mysid shrimp (*Americamysis bahia*). The evaluations involved a comparison of standard USEPA protocols to a slightly modified exposure method using two common stormwater-associated toxicants, copper and zinc. Tests were performed under static (96-h continuous exposure) and pulsed exposures (time varying toxicant exposure. Following pulsed exposures, organisms were transferred to uncontaminated seawater for the remainder of the 96-h exposure period. To date, results with these species and endpoints suggest pulsed exposure median effective and median lethal concentrations are up to two orders of magnitude greater (i.e. less sensitive) than current recommended continuous approaches for these test methods. Additional considerations assessed during these studies included: (1) appropriate timing of the onset of a pulse during laboratory exposures – time zero or after an acclimation period in receiving water; (2) evaluation of different life stages of proposed test organisms; and (3) the potential for latent effects following the proposed pulsed exposure duration. This presentation will summarize results to date, and future plans to assess proposed Whole Effluent Toxicity test modifications within National Pollutant Discharge Elimination System requirements for episodic discharges.

### 643 A Multiple Line of Evidence Approach to Assess Stormwater Impacts from the Scripps Institution of Oceanography to Receiving Waters in San Diego, CA

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The variable nature of stormwater runoff presents unique challenges with regard to accurately characterizing impacts to receiving waters.

A multiple line of evidence approach is needed to better characterize potential biological impacts and stressors of concern. The University of California's Scripps Institution of Oceanography (SIO) is situated on the Pacific Ocean adjacent to an Area of Special Biological Significance. Seawater from the research facilities and a public aquarium, as well as stormwater during wet weather, enters the ocean off SIO requiring an NPDES permit to monitor discharges from the facility during both dry and wet weather. Routine monitoring includes a suite of chemicals and toxicity tests in samples from several outfalls and the receiving water twice annually, and a biological community assessment and bioaccumulation study once every 5-years. To better understand impacts in the adjacent receiving waters, in situ toxicity tests, toxicity identification evaluations (TIEs), and a variety of other special studies have been integrated into the monitoring program over the past 12 years. Most recently, in 2018, a series of bioassays which altered the duration of the stormwater pulse (6, 26, and 96 hours) were also performed using the purple sea urchin *Strongylocentrotus purpuratus*. Although there are chemical constituents of potential concern in undiluted storm water runoff and toxicity has been observed in stormwater samples collected at the end of pipe, rapid mixing and dilution is evident in the immediate marine receiving waters. However, long term monitoring at this site has demonstrated limited to no toxicity observed in receiving water samples in the lab or in situ during wet weather. TIEs have identified copper and zinc as toxicants in stormwater at the end of pipe during wet weather, and toxic algae blooms as a source of toxicity in the receiving waters during a few dry weather events. The pulsed study found that duration of exposure did affect the outcome, with shorter exposures showing less of an effect. By reviewing the results of multiple lines of evidence collectively over time these enhanced special studies have provided a greater understanding of the impacts of wet and dry weather runoff to a dynamic marine receiving environment. This has allowed for better prioritization of BMPs, and has also shed light on the need to consider alternative approaches for assessment of episodic discharges such as stormwater runoff.

**644 Using integrative passive sampling devices to obtain more meaningful and cost-effective data on metal-associated impacts from stormwater runoff**

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In many cases, stormwater compliance monitoring is labor intensive, expensive, and largely unsuccessful in providing the data needed to support stormwater management goals. To help address these issues, the time-integrative, heavy metal passive samplers, Diffusive Gradients in Thin film (DGTs), are being used to monitor stormwater runoff for copper, nickel, lead, and zinc. DGTs were co-located with traditional autosamplers within the stormwater conveyance systems at Naval Base San Diego (NBSD) to provide a direct comparison with composite sampling. In a more controlled fashion, DGTs were exposed in the laboratory to composite samples from NBSD stormwater conveyance systems. These controlled experiments showed increasing uptake over time (range = 1.5 to 24 h) for copper, cadmium, and nickel, with statistically significant positive, linear correlations ($r^2$>0.980) between time exposed and metal mass accumulated. However, it appears that the corresponding calculations of the DGT-labile fraction relative to the dissolved fraction fluctuated across the different exposure durations. In general, trends observed for DGT-labile measurements from the field were consistent with trends seen in the lab DGT exposures and traditional dissolved metal measurements from composite samples. Finally, time-weighted, average concentrations of copper and zinc from DGTs that were deployed for the first half and second half of a storm event were within 30% of measurements from DGTs that were deployed for the entire storm event in the same stormwater basin. Cumulatively, these results show promise for using continuous monitoring with DGTs as an approach that produces concentrations more representative of those in the receiving environment during episodic events than those concentrations measured from traditional grab or composite chemistry sampling.

## Non-Conventional Exposure Routes and Transport Media of Consumer Product Chemicals to Improve Environmental Policy

**645 High-throughput exposure modeling for 8000 Tox21 chemicals in the environment and in consumer products: Identification of dominant pathways**

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Near-field human exposure to chemicals in consumer products gains importance, since there is growing evidence that near-field chemical intakes can dominate overall exposures. However, exposure and toxicity characterization models used in life cycle impact assessment and comparative risk assessment are usually limited to chemicals emitted to the far-field environment. Thus, we developed a matrix-based framework based on the widely used USEtox model to integrate near- and far field chemical exposures and to identify dominant pathways. In this framework, the transfer rate constants are converted to direct transfer fractions from emission to receiving compartments. We connected near-field compartments including product, near-person air, with far-field compartments (e.g. air, water, soil), via wastewater and solid waste treatment plants. By inversion of the transfer fractions matrix, cumulative transfer fractions from products to various human receptors are obtained, also known as product intake fractions (PiFs), defined as the fraction of a chemical used in a product application that is cumulatively taken in by various receptors during use and disposal stages. The direct transfer fractions from different product and emission scenarios are calculated by 7 underlying models (skin surface layer, object surface layer, article interior, indoor air, food contact material, pesticide, direct environmental emission). Our framework was applied in batch mode to generate high-throughput exposure estimates of the PiFs for 8167 chemicals in Tox21 database via four pathways: near-field (residential), dietary, far-field industrial, and far-field pesticide. The total PiF summing users and non-users (i.e., general population) across all exposure pathways for the 8167 chemicals ranges from 1.9E-4 to 0.34, from 4.8E-6 to 0.19, and from 6.3E-6 to 0.22 via residential, dietary and far-field industrial pathways, respectively. In general, the residential pathway is associated with the highest total PiF, highlighting the importance of near-field chemical exposures. For semi-volatile compounds, dominant pathways include non-conventional pathways via dust, dermal gaseous uptake and mouthing for children. Results will also be presented for stochastic modeling of individual doses for another 9000 product-chemical combinations. The proposed tool is adequate for quantitatively informing exposure assessments for Chemical Alternatives Assessment, Life Cycle Assessment and Risk assessment.

**646 New Insights of Human Exposure to Consumer Product Chemicals from Comprehensive Chemical Profiles of Indoor Dust**

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Comprehensive chemical profiles of indoor dust may help better understand residential chemical exposure. Due to the wide range of chemicals found in consumer products, it is not easy to determine the full range of exposures to compounds emanating from consumer products in the home. SVOCs released from their original sources are fairly persistent indoors, and thus redistributed over time and partitioned to indoor air, settled dust, and other indoor surfaces. Dust is therefore an ideal media for identifying comprehensively what compounds are used in the home. Furthermore, for SVOCs with low vapor pressures, the levels in indoor dust are known to serve as an alternate marker of chemical exposure with common indoor sources (e.g., furniture, plastics, PCPs, pesticides, etc.). This study presents concentrations of a broad spectrum of semivolatile organic compounds (SVOCs) in household dust measured via a comprehensive analytical workflow. We quantified SVOCs in dust samples collected from 38 California homes by applying a combination of target, suspect, and non-target screening approaches to liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF/MS) and gas chromatography (GC)-QTOF/MS. A total of 257 compounds were detected in at least one sample and 170 compounds were detected in more than 50% of the samples. Of the 257 compounds, 168 compounds were unambiguously confirmed and quantified by reference standards and 135 compounds were detected for the first time in U.S. household dust. Median concentrations ranged from 5 to ~100,000 ng/g of dust. We utilized in vitro toxicology tests to identify newly detected compounds in our dust that have endocrine disrupting and neurotoxic potential. These identified compounds should further be studied in the context of consumer products, as they are most likely entering the home from consumer product use, and serves to broaden the landscape of chemicals that should be considered. This study demonstrates a novel analytical workflow to detect a large number of *known* and *unknown* SVOCs in indoor dust. Our results provide a comprehensive picture of the chemical fingerprint in dust which can be used in better understanding residential chemical exposure and associated adverse health effects.

**647 Our e-devices: Can't live without them but how are they contributing to our exposures to flame retardants?**

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Electronic products are pervasive in just about everyone's lives, including children. They contain a wide array of hazardous chemicals including flame retardants. Their relatively short lifespan ensures a ready supply of e-waste that, in many jurisdictions, is mandated for handling in an environmentally sound fashion. We investigated electronic devices as sources of flame retardant exposure during their use and End-of-Life phases. In a residential exposure study of 51 women in Toronto and Ottawa, Canada, we measured concentrations of organophosphate esters (OPEs) and halogenated flame retardants (HFRs) in air and floor dust in their homes, on their hands, and the surfaces of their electronic devices. Also, 8 OPE metabolites were analyzed in the urine samples of 44 participants. Surface concentrations of OPEs and HFRs were significantly higher on handheld electronic devices (e.g., cell phone, tablet) than non-handheld devices (e.g., TV, desktop), except for BDE-209 and DBDPE. OPE concentrations on hands were most frequently correlated with concentrations on cell phones, whereas PBDEs, EHTBB and BEHTBP on hands were most frequently correlated with concentrations on laptops. ∑OPE concentrations on participants' cell phones were significantly correlated with levels of the corresponding metabolite in urine samples, driven mostly by TPhP. We also found that levels of TDCPP in dust and cell phones were significantly correlated with the corresponding urinary metabolite. The data suggest that cell phones are both sources of OPEs as well as indicators of exposure of OPEs and some HFRs. In an e-waste study in Ontario, Canada, we also measured concentrations of OPEs and HFRs in air and floor dust. Levels of BDE-209 were by far highest, followed by OPEs. Compounds in this dusty environment were found in inhalable particles. Total exposure was dominated by dust ingestion followed by inhalation. These exposures were relatively high and, in some cases, exceeded those working in the informal recycling sector of low income countries. Some exposures also exceeded reference doses. Electronic devices have profoundly changed our society. When assessing benefits and risks, we need to add the adverse effects arising due to chemical exposures from these devices during the use and End-of-Life phases and seek solutions to minimize those effects. The environmental consequences of our widespread use of electronic devices are even farther reaching.

**648 Exposure of Contaminants of Emerging Concern (CECs) and Other Chemicals in Urban Stormwater Runoff in the Conterminous United States**

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Stormwater runoff in urban areas can contain a variety of chemical constituents, including contaminants of emerging concern (CECs) (e.g. pharmaceuticals, new-use pesticides, hormones, plasticizers, fire-retardants, fragrances, and personal-care products), that enter into surface waterbodies and infiltrate to groundwater. CECs are present in water resources on a global scale with mounting evidence of potential deleterious effects to both aquatic and terrestrial organisms. Green infrastructure (GI) is an emerging technology that is intended to supplement natural environmental processes with engineered systems to enhance stormwater infiltration into the subsurface. Municipalities and water management agencies across the United States are increasingly using GI at various scales to recharge groundwater, reduce stormwater volumes, and minimize contaminant loads to receiving surface waterbodies. Additional research is needed to provide accurate information on the chemical compositions and concentrations of CECs, and potential water-quality effects on groundwater from infiltration of stormwater using GI. As part of a national-scale study to determine the chemical compositions and concentrations of trace organic and inorganic chemicals in urban stormwater from a range of watershed sizes and landuses, 57 stormwater samples were collected from 21 field sites across 17 states. Targeted chemicals included 476 trace organics (e.g. halogenated chemicals, hormones, halogenated chemicals, methylmercury, pharmaceuticals, pesticides,) and 64 inorganic (e.g. anions, cations, total mercury, trace and rare-earth elements) constituents. Stormwater samples were collected before flowing into surface waterbodies or infiltration to groundwater via GI (e.g. rain gardens, ecological recharge basins, spreading grounds). Preliminary results indicate that organic chemicals are ubiquitous in urban stormwater. Watershed characteristics (e.g. topography, landuse, climate, geology, etc.) and storm event hydrology will be used to interpret the complete set of chemical data.

**649 Nontarget screening for organic contaminants in wastewater at a sub-sewershed scale to identify spatial and temporal patterns**

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Trace organic constituents (TOCs) such as pesticides and pharmaceuticals have been identified in wastewater entering treatment facilities, creating concern due to their poor removal by conventional wastewater treatment and toxicity potential in receiving environmental waters. However, there is little source information for these TOCs on a sub-sewershed scale. This study examines 24-h time-weighted composite samples collected monthly from February through December of 2016, at the wastewater treatment plant influent, effluent, ten sub-sewershed laterals, and three locations expected to have high pesticide loads (i.e. pet groomer, pest control operator, and laundromat). All samples were analyzed using both liquid and gas chromatography time-of-flight mass spectrometry (LC-QTOF-MS and GC-QTOF-MS) to comprehensively screen for roughly 3500 suspect chemicals. LC-QTOF-MS data were acquired using electrospray ionization (ESI) in positive and negative modes with All-Ions fragmentation, and two accurate mass MS/MS libraries containing >2000 chemicals were screened. The extracts were run on GC-QTOF-MS in electron impact (EI) mode using a retention time locked method and the spectra were screened against a pesticide library containing exact mass fragments and retention times. This study focusses on both the ways in which TOCs in the sewershed vary with time and space, and the contributions of residential, industrial, commercial, institutional, and municipal sources.

**650 Occurrence and Sources of Pesticides to Urban Wastewater and the Environment**

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Municipal wastewater has long been recognized as a pathway for discharge to receiving waters and the environment of contaminants derived from pharmaceuticals, personal care and cleaning formulations, and other consumer products. However, relatively few studies have evaluated this pathway for current-use pesticides. A state-of-the-science overview of the occurrence and fate of pesticides in wastewater, both before and after treatment, indicates this pathway is significant and should not be overlooked. A comprehensive conceptual model will be presented to establish all relevant pesticide use patterns with the potential for down-the-drain transport, both through direct wash-off and indirect contact resulting in subsequent wash-off. Case studies will highlight the importance of neglected sources of environmental contamination via the wastewater pathway. Recommendations for future monitoring and modeling studies to inform source control will be provided based on analysis of key data gaps.

**651 Estimating environmental emissions and aquatic fate of sludge-bound CECs using spatial modeling and US datasets**

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In the US, 50% of the sludge produced during wastewater treatment is recycled to land (www.epa.gov/biosolids). Some chemicals in consumer products may be highly removed during the wastewater treatment process due to sorption and binding to organic matter, ending up in sludge solids where it has the potential to be applied to land surfaces, subject to erosion or runoff processes potentially entering nearby surface waters. However, biosolids mass applied to land is not evenly distributed across the US landscape due to variable population density, local sludge management practices, and availability of land application sites. We have developed a proof-of-concept model to aide in the prospective assessment of CECs contained in WWTP sludge applied to land. This spatially-explicit, national model is based on publicly available datasets, combined with a spatial-hydrologic framework containing geographically variable emissions linked to a river network allowing for environmental transport via surface water. The hydrologic framework is based on a set of basins and rivers (www.hydrosheds.org) linked to emission characteristics for over 77,000 sub-basins. Emission characteristics are derived from facility data in the USEPA Clean Watersheds Needs Survey (www.epa.gov/cwns) to estimate consumer product usage linked to wastewater treatment, and spatially-variable data on biosolid applications. The USDA Cropland Data Layer (www.nass.usda.gov) provides potential land application sites, from which proximity to surface water plays a role in the potential for CECs to transport from land to freshwater (using a meta-model estimated from pesticide assessment models). Concentrations of CECs are routed through the river network based on local river attributes (e.g., flow) combined with assumptions about chemical fate in the aquatic environment. Results of various simulations show the spatial patterns of biosolids applications, potential to enter surface water, and estimated freshwater concentrations of an ingredient in a hypothetical consumer product. Implications of altering model assumptions are discussed. While the presented material is a simulated example of the environmental emission and fate of a consumer product ingredient, it represents a viable approach to assessing whether this pathway via land applied biosolids may be of concern for consumer product chemicals, and ultimately helping to inform environmental policy on this subject.

**652 Environmental Conditions Affecting Re-release of 4-Nonylphenol into an Aqueous Medium**

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4-Nonylphenol (4NP) is a persistent organic pollutant with endocrine disrupting properties. This non-polar product of microbial degradation is derived from the surfactant nonylphenol polyethoxylate (NPE). 4NP is capable of long range transport from its point of application. The Sierra Nevada Mountains (SNM) divide the agricultural Central Valley from the pristine eastern slopes. Bioactive concentrations of 4NP have been found in the surface water, soils, snow and particulate matter of the Eastern SNM. Seasonal snow pack concentrations can be 20 to 100 times higher than in surface waters, and 4NP was detected in snow samples from the Palisade Glacier. Accelerated glacial ablation may result in a significant release of 4NP from particulates in glacial melt water. To determine whether 4NP will re-release from particulates into glacial melt water, batch desorption assays were run on particulate matter dosed with 4NP. Desorption was measured in 63, 125, 250 and 500 mm particles under two different temperature conditions and with varying fractions of organic carbon (OC). Water turbulence effects on the fraction of 4NP released were also investigated. Lower temperature slowed the release rate of 4NP from particulates, but at equilibrium the amount released was statistically the same. Similarly, turbulence has little effect on the total percent of 4NP released. There was a statistically significant correlation ($r = 0.72$) between the percent of organic carbon in the particulate matter and the concentration of bound 4NP found in environmental samples. However, particulates with higher percentages OC (80% or more) released very little of the bound 4NP (0.003%) compared to particulates containing 4-5% OC, which released up to 15%. Particle size had the most significant effect on the release of 4NP. Smaller particles (< 63 mm) released up to 35% of bound 4NP, all other things being held constant, versus particles 500 mm or larger, which may release as little as 0.1 to 1%. This may be attributable to surface adsorption in smaller particles versus surface and pore space absorption in larger particles. Glacial flour, the fine particles created as glaciers grind rocks to powder, can be smaller than 1 mm in size. Water and sediment samples taken from below the Palisades Glacier showed the greatest 4NP concentrations at the lakes directly below the glacier. This implies that glacial flour is likely to release a high percentage of any adsorbed 4NP.

## Water and Land Management to Reduce Methylmercury Bioaccumulation

### 653 Effect of organic matter concentration and characteristics on mercury mobilization and methylmercury production at an abandoned mine site

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Thousands of abandoned mines throughout the western region of North America contain elevated total-mercury (THg) concentrations. Soil amendments that promote vegetation growth on mine tailing may reduce erosion of high Hg content particles that can be transported to downstream waterbodies. Many of the mine sites exhibit minimal vegetative cover due to a lack of nutrients and organic carbon in the tailings and are susceptible to erosion. Application of organic soil amendments is a proven technique to promote vegetation growth on mine tailings and reduce erosion. However, in the case of Hg, organic matter additions have the potential to exacerbate Hg contamination by simulating microbial populations capable of producing the more toxic and bioaccumulative organic form of Hg (methylmercury—MeHg). The project's objectives were: 1) determine the mechanism by which Hg is mobilized from an abandoned mine site (Cinnabar Mine, ID), and 2) identify how variations in organic matter additions and quality (assessed through excitation emission matrix fluorescence spectroscopy —EEMs) affect MeHg production in mine tailings. Annual loads of filtered-passing and particulate THg at a downstream gaging station were modeled using LOADEST (n=34). To assess MeHg production, stable isotope methylation assays and mesocosm experiments were conducted using different types of commercially available organic matter soil amendments to mine tailings. The results showed that >80% of the THg mobilized from the mine was bound to particles and >90% of the annual loading occurred during the period of elevated discharge during spring snowmelt. Methylation rates varied between different types of soil amendments and were the most strongly correlated with the EEMs T and N peaks. The mesocosm experiments showed that under anoxic conditions carbon amendments to tailings could significantly increase porewater MeHg concentrations (up to 13 ±3 ng/L). In addition, the carbon amendments significantly increased THg partitioning to the porewater. Overall, these results indicate that efforts to reduce surface erosion at abandoned mine sites could be effective at reducing Hg mobilization with particles to downstream waterbodies; however, some types of soil amendments have the potential to significantly increase Hg methylation as well as THg partitioning to porewater.

### 654 In-situ coagulation systems can lower wetland mercury export and bioaccumulation

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Coagulation is commonly used to remove constituents from water, including dissolved organic matter (DOM) and suspended particulate matter (SPM), via the formation of larger particles (flocculate, or floc) which settle out of the water column. Because mercury (Hg) and methylmercury (MeHg) are predominantly bound to DOM and SPM, coagulation may be effective in controlling their fate and transport. In a series of Hg-related studies, our USGS team demonstrated that additions of metallic and chitosan-based coagulants to a variety of surface waters promotes removal of Hg and MeHg. Laboratory studies showed that coagulation effectively traps Hg and MeHg associated with DOM and fine, suspended particulates in larger aggregates and (or) denser particulate forms that settle out. Once deposited, the sedimentary materials largely remain intact and are relatively stable. Field-based studies conducted in the Sacramento-San Joaquin Delta and the Cache Creek watershed confirmed the overall effectiveness of Fe- and Al-based coagulants for removal of Hg and MeHg from both DOM- and particle-bound Hg species, whereas chitosan-based coagulants were effective for the removal of Hg and MeHg adsorbed to SPM only. Stability studies following deposition showed that a portion of the Hg in the settled floc was subsequently released under anaerobic conditions, whereas under aerobic conditions, Hg and MeHg were released into the dissolved phase as floc degraded. THg concentrations in the overlying water were higher for the Fe-based treatment than for the Al-based treatment and the (untreated) control, but the converse was true for MeHg because new MeHg production was inhibited by the Fe-based treatment. The Al-based treatment produced larger and more physically stable flocs than either Fe-based or chitosan-based treatment. Overall, less than 5% of THg and MeHg removed via coagulation and settling was subsequent released from settled materials under the conditions tested. Furthermore, the Fe-based treatment was more effective than the Al-based treatment for decreasing Hg bioaccumulation in fish. In general, coagulation was broadly effective for transferring Hg and MeHg from water to sediment, and treated wetlands show a net benefit for minimizing THg and MeHg transport (relative to control wetlands). Overall, Fe-based treatments appear to be more effective at reducing water-column Hg concentrations and bioaccumulation than Al-based treatments.

### 655 Seasonal and Redox-Mediated Patterns of Methylmercury Release from Profundal Sediment in Hodges Reservoir, California

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The San Diego Public Utilities Department will install an 8-ton per day hypolimnetic oxygenation system to enhance bottom water quality in Hodges Reservoir, a hypereutrophic reservoir near San Diego, California. The primary aim of the oxygenation system is to lower internal nutrient loading. But with the implementation of the State Water Board's Statewide Mercury Control Program for Reservoirs, there is also interest in assessing if oxygenation will repress the buildup of toxic methylmercury (MeHg) in anoxic bottom water. We implemented a laboratory and field monitoring effort to assess nutrient and metal cycling in the profundal zone under differing redox regimes. Replicate bench-scale sediment-water interface chambers (~2 L) containing profundal sediment were incubated under oxic and anoxic conditions. Chamber water overlaying sediment was monitored for several redox-sensitive parameters including MeHg, phosphate, ammonia, manganese, and sulfide. In general, maintenance of a well-oxygenated sediment-water interface repressed sediment release of these compounds. Typical oxic and anoxic fluxes were < 5 versus 20-40 mg-P/m$^2$·d for phosphate, < 5 versus 60-140 mg-N/m$^2$·d for ammonia, < 1 versus 50-60 mg/m$^2$·d for manganese, and < 20 versus 150-300 ng/m$^2$·d for MeHg. Sulfate uptake positively correlated ($R^2 = 0.62$, $P < 0.02$, n = 8) with MeHg efflux in chambers early in the anoxic phase, but not in the late anoxic phase when MeHg efflux dropped dramatically. This drop may be attributed to a decrease in Hg(II) bioavailability associated with sulfidic conditions or enhanced demethylation associated with methanogenesis. At the field scale, water quality monitoring in 2017 mirrored experimental results. Bottom water anoxia was associated with hypolimnetic buildup of redox sensitive compounds. Buildup of MeHg (~1 ng/L) in bottom water showed a seasonal cycle, increasing in the summer but then decreasing in the early fall, coincidental with significant sulfide accumulation (~ 25 mg/L). Both experimental results and field monitoring suggest that in eutrophic reservoirs, there is an early seasonal window of moderately reduced redox that is optimal for MeHg production in the profundal zone. Results also suggest that incomplete oxygenation of the profundal zone could inadvertently enhance MeHg buildup by shifting the profundal zone from highly reduced conditions to mildly reduced conditions that favor MeHg production.

**656 Mercury bioaccumulation through a semi-arid river-reservoir system: Effects of reservoir structure and habitat type**

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Hydrologic alteration is a defining ecological stressor to aquatic habitats associated with the Anthropocene, particularly in arid environments. Reservoir creation and subsequent management can have substantial influences on water quality, food web structure, and habitat availability. Additionally, reservoir creation has been shown to increase methylmercury production and bioaccumulation in newly inundated systems. However, relatively less is known about mercury bioaccumulation in established reservoirs or in contrast with other aquatic habitats. Paring studies at two different scales, we evaluated how Hg bioaccumulation varies among habitats and through reservoir food webs. First, we examined differences in fish mercury concentrations between free-flowing and impounded segments of the Snake River in Idaho, USA, a semi-arid river with 22 impoundments constructed between the early 1900's and 1980's along its course. Next, in a three-reservoir complex within the system we examined spatial and temporal variation of mercury transfer through the food web, and used mercury isotopes to examine potential source attribution. Along the Snake River, preliminary results indicate that fish mercury concentrations in reservoirs (0.141 ± 0.003 µg/g ww) and river segments directly below reservoirs (0.143 ± 0.005 µg/g ww) are 1.7- and 1.8-fold higher, respectively than in free-flowing segments not directly below reservoirs (0.082 ± 0.003 µg/g ww). Further, in free-flowing river segments, bass THg concentrations declined with distance from the nearest upstream dam, suggesting that these reservoirs can influence mercury bioaccumulation in fishes far downstream. Within reservoirs, methylmercury concentrations at the base of the food web were 2.3-fold higher at stratified locations in comparison to oxygenated, mixed habitats. Additionally, small prey fish mercury concentrations closely matched the spatial patterns of lower food web organisms, whereas top predator fishes increased by 3.3-fold through the linear extent of the complex. Cumulatively, our findings are indicate that reservoirs play an important role in mercury risk of aquatic ecosystems, but as highly managed habtats may also provide unique opportunities to mitigate this risk.

**657 Implications of freshwater mussels on mercury cycling and bioaccumulation in fish**

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Mercury is a globally important contaminant which is of particular concern in stream systems. Because the consumption of contaminated fish is the most significant source of mercury (Hg) exposure to humans, regulatory guidelines that focus on the protection of human health require monitoring of both aqueous and fish tissue Hg concentrations at contaminated sites. However, because Hg is predominantly accumulated in fish via dietary rather than aqueous exposure, the link between aqueous Hg concentrations and concentrations in fish is not always straightforward, confounding remediation efforts at contaminated sites. Benthic macroinvertebrates can often comprise a major portion of fish diets and can therefore be important in the trophic transfer of bioaccumulative contaminants like Hg to fish. In particular, freshwater mussels, because they filter particulates (and dissolved nutrients) from the water column, can affect mercury bioaccumulation throughout the food web by exerting effects on periphyton, dissolved organic matter, methylating bacteria, and dissolved mercury concentrations. The conservation of mussel populations is increasingly recognized to be a critical need, because they are among the most threatened group of organisms globally and because they are keystone species in riverine systems, providing multiple ecosystem services. Here, we examine the potential implications of re-introducing native freshwater mussels into East Fork Poplar Creek, a Hg-contaminated stream in East Tennessee. We combine results from laboratory experiments and field deployments to quantify filtration and mercury uptake rates in four species of mussels (Utterbackia imbecillis, Villosa iris, Lampsilis ovata, and Lampsilis fasciola) under varying environmental conditions and model effects on mercury bioaccumulation in fish. Our results indicate that filtration by freshwater mussels can have a significant impact on mercury concentrations and bioaccumulation in stream food webs.

**658 Quantifying the effects of activated carbon amendment and tidal inundation on mercury and methylmercury partitioning in Phragmites marsh mesocosms**

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Activated carbon (AC) has been proposed as an in situ remediation method for mercury (Hg) and methylmercury (MeHg) in ecologically sensitive environments. A limited set of field trials and laboratory experiments have shown that AC amendments increase the partitioning of Hg and MeHg to the solid phase, decreasing porewater concentrations and bioaccumulation in benthic organisms. However, available data also suggest that AC efficacy varies with site geochemistry, and more data are needed to determine which environmental parameters dictate AC efficacy. With this study, we used field mesocosms to investigate the effectiveness of AC as an in situ amendment in Hg and MeHg-contaminated soil from the Berry's Creek wetland in New Jersey, USA. The objectives of this study were to: (1) assess the impact of AC on MeHg and Hg concentrations in porewater in *Phragmites* marsh mesocosms; (2) to evaluate the impact of tidal elevation on the efficacy of the AC amendment; and (3) to determine the impact of AC on Hg and MeHg partitioning into the solid phase. *Phragmites* marsh mesocosms were constructed from large-diameter, capped, PVC pipes and set at two different elevations in a tidal creek. Three types of treatment mesocosms were constructed: Control, 5% dw AC-amended, and 9 % dw AC+$MnO_2$-amended. The $MnO_2$ was added to poise redox potential at manganese reduction. Mn(IV) is a more energetically favorable electron accepter for bacteria than iron or sulfate, and an excess of Mn(IV) could shift the microbial community away from sulfate and iron reduction, reducing the population of Hg-methylating bacteria. Mesocosm porewater was monitored over four months. Sediment cores were taken at the conclusion of the experiment. The 9% AC+$MnO_2$ treatment decreased porewater Hg by ~85% and MeHg ~75X% relative to controls, while the 5% AC treatment decreased Hg by ~40%, and MeHg by ~25% (the latter was not a significant reduction). Mesocosm elevation significantly impacted redox chemistry in porewater and soil, but no significant difference was seen in Hg and MeHg porewater concentrations. Both the 5% AC and 9% AC+$MnO_2$ treatments significantly increased Hg and MeHg partitioning to soils. There was no significant difference between treatments for soil Hg concentration, but both amendments significantly increased soil MeHg concentration. These results contribute to our overall goal to develop an empirical model to predict the efficacy of AC for Hg and MeHg remediation across ecosystems.

**659 Investigations on the sources of Yolo Bypass floodwater methylmercury production**

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The State of California, Central Valley Regional Water Quality Control Board (Board), adopted amendments to its Basin Plan in 2010 to address mercury (Hg) and methylmercury (MeHg) impairment in the Sacramento-San Joaquin Delta (Delta), the largest estuary on the west coast. Previous studies have demonstrated that in some months the Sacramento River contributes between 50 and 85 percent of the MeHg to

the Delta. However, during major flood events, the Yolo Bypass produces approximately 40% of all MeHg exported from the Sacramento basin. This is surprising because the Yolo Bypass, a 59,000-acre flood conveyance system designed to divert Sacramento River storm water around the City of Sacramento, is 285 times smaller than the 16,765,000 acre Sacramento Basin. The Board is requiring that total MeHg loads from the Yolo Bypass be reduced from 100 g/yr to 22 g/yr, a 78% reduction. The State of California, Department of Water Resources (DWR) is required to evaluate whether operational changes or other management strategies could be implemented to reduce open water MeHg production. As part of this mandate, DWR, together with collaborating agencies, are investigating if Best Management Practices (BMPs) are available to mitigate MeHg export from the Yolo Bypass. However, BMP recommendations require an understanding of the dominant sources contributing to the observed MeHg in-bypass production. This presentation focuses on a series of laboratory, mesocosm, and field studies investigating the relative contributions of erosion, pore water flux, and senescing vegetation inundated by flood waters to in-bypass production. Our scaled-up load calculations from different land uses for erosion and pore water flux suggests that while these sources may be important contributors to MeHg load under small and localized flooding events, they cannot account for observed in-bypass production under the large flooding events associated with export of MeHg from the Yolo Bypass. Since pasture lands are the largest land use in the Yolo Bypass, our investigations are focusing on the role vegetation could play in-bypass production. Our flooded mesocosm pilot experiments, incubated for 35 days under ambient winter conditions, showed that MeHg production differed significantly between disked and vegetated Yolo Bypass pasture lands. Follow-up experiments are in the design stage to further refine whether vegetation reduction can be used as a BMP to limit MeHg production in the Yolo Bypass.

**660 Importance of habitat to methylmercury distribution in water, sediment, and biota in a managed floodplain, Northern California**

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The Cache Creek Settling Basin (CCSB) is a 1600-hectare floodplain area in Yolo County, California (USA) bounded by levees since its construction in 1937 and expansion in 1993. The CCSB traps sediment to reduce deposition in the Yolo Bypass, a conveyance for Sacramento River flood waters, that protects the city of Sacramento. The CCSB traps particulate mercury (Hg) from the Cache Creek watershed, which has several natural and anthropogenic Hg sources: wastes from inactive Hg mines and gold mines (where Hg-amalgamation was used historically), mineral springs, and erosion of geologic materials with naturally elevated Hg. Load calculations based on water sampling during 2010–15 indicate: 62% ± 7% of particulate total Hg (THg) was trapped in the CCSB, with no net change in filtered-water THg load; 60% ± 10% of particulate methylmercury (MeHg) was trapped, whereas filtered-water MeHg load increased 38% ± 26% in basin outflows relative to inflows. To investigate MeHg gradients within the CCSB, four principal habitats were mapped: open water (stream channels and canals), riparian (along stream channels), non-agricultural floodplain, and agricultural (row crops, primarily corn and tomato). In shallow sediment (0–2 cm) collected at 93 locations within the CCSB during 2010–17, agricultural and open-water habitats had consistently lower MeHg than riparian and non-agricultural floodplain habitats, and lower total reduced sulfur (TRS) and organic matter. Within each habitat, there was an increase along the direction of stream flow in sediment %MeHg (100*MeHg/THg). TRS and organic matter also increased in the flow direction, and median grain size decreased. THg in tissue of western mosquitofish (Gambusia affinis), for caged (30-day exposure) and wild samples, and in water samples collected at the fish-cage sites also increased with downstream distance. THg in house wren (Troglodytes aedon) eggs increased with downstream distance during the wetter years but not the drier years of the study. Sediment MeHg was higher when the basin was flooded, whereas reactive Hg(II), a precursor to MeHg, was higher during drier periods. The finding that riparian and non-agricultural floodplain habitats had significantly higher %MeHg than agricultural (row-crop) and open-water habitats (by a factor of about 2) could have broad implications for resource management, given that these habitats are typical of managed floodplains in California and elsewhere.

## Soil Contaminants: Fate, Bioavailability, Environmental Toxicology in Ecological and Human Health Risk Assessment

**661 Recommendations for Sieving Soil and Dust Samples at Superfund Sites for Assessment of Incidental Ingestion Exposures**

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Recommendations for sieving soil and dust samples at Superfund sites for assessment of incidental ingestion via dermal adherence Incidental ingestion is the primary pathway for exposure to lead, and other contaminants, in soil and dust. The current understanding of this exposure pathway assumes that incidental ingestion occurs subsequent to (and is dependent on) dermal adherence. Hence, site-specific risk assessment requires that soil and dust samples be sieved to accurately represent incidentally ingested material that adheres to skin. Reliable data on the particle size fraction that is most likely to adhere to hands, and on the lead concentration found in that particle size, can improve the accuracy of exposure and risk calculations in lead risk assessments. In response to this data need, the EPA has prepared guidance for sieving soil and dust samples for assessment of incidental ingestion. The focus of the guidance is on lead-contaminated sites, but is also applicable to assessments of exposure to other contaminants in soil or dust. The guidance document contains a review of the current data on the relationship between particle size fractions sieved at lead-contaminated sites and the likelihood that they will adhere to hands and be incidentally ingested. The existing sieving guidance, developed by EPA's Office of Land and Emergency Management (OLEM) in 1994, recommended the

**662 Bioavailability of Arsenic in Surface Soil from Historical Herbicide Application**

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Determining a site-specific relative bioavailability (RBA) value for a chemical in soil allows for the development of a site-specific risk-based remedial goal that is protective of human health but not unnecessarily conservative. An assessment of the RBA of arsenic in surface soil was conducted for a site in northern California impacted by historical application of arsenical herbicides. Detected concentrations of arsenic at the site range from below the background level of 10 milligrams per kilogram (mg/kg) to 240 mg/kg and a human health risk assessment indicated arsenic in surface soil (0-0.5 foot) poses a risk to future residential receptors. This bioavailability study, conducted prior to the recent releases of state and federal guidance for in vitro testing, included paired in vivo (swine) and in vitro tests to identify the method(s) appropriate for further in vitro testing of additional samples. Relatively low variation in the predicted RBA was reported for thirty-seven soil samples collected from locations throughout the site with arsenic concentrations that ranged nearly two orders of magnitude in concentration (from 1.97 to 164 mg/kg). A site-specific RBA of 39.5 percent was selected based on the in vivo tests, the results of which were similar to the sitewide in vitro tests.

**663 California Arsenic Bioaccessibility Method: Recommended Method for bench-Top Prediction of Relative Bioavailability in Contaminated Soils**

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The California Department of Toxic Substances Control (DTSC) Human and Ecological Risk Office (HERO) recently released guidance on evaluating arsenic bioavailability in contaminated soils. Human Health Risk Assessment (HHRA) Note 6 recommends using the recently published California Arsenic Bioaccessibility (CAB) in vitro method to predict in vivo relative bioavailability (RBA). Traditional HHRAs assume that arsenic in soils is 100% bioavailable. However, it is well known that this is not typically the case; USEPA recommends a default RBA of 60% in the absence of site-specific data. Until recently this meant using an in vivo animal study, which are both expensive and time consuming. CAB is a quick and inexpensive method, which allows for a more efficient use of the resources available for the cleanup while maintaining a high level of health protectiveness. CAB is highly predictive of arsenic RBAs for a wide variety of soil types when the total arsenic concentrations are less than 1,500 mg/kg, but tends to over-predict in soils with higher levels of arsenic. Consequently, DTSC is recommending the use of CAB to predict site-specific RBAs of arsenic in soil at most sites under its jurisdiction. HHRA Note 6 provides guidance on when and where it is most advantageous to conduct a bioavailability study using CAB and how the results of such a study should be incorporated into a risk assessment. Important considerations include the naturally occurring background concentrations of arsenic, the range of arsenic due to contamination, the planned future land use of the site, and community acceptance. Risk-based concentrations for arsenic are typically lower than naturally occurring background levels in soils. HHRA Note 6 provides guidance on how to determine clean-up values under multiple outcome scenarios. DTSC HERO toxicologists are available for consultation on applying this guidance on a site-specific basis and have a long-term goal of creating a database of all sites in the State that have used CAB in their decision making process. This database would be publically accessible with the goal of helping to inform future site evalutions.

**664 Predicting Arsenic Bioavailability in Contaminated Soils by Using In Vitro Gastrointestinal Bioaccessibility and Speciation**

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Exposure risk associated with soils contaminated with As is assessed by human health risk assessment (HHRA). A more accurate and site-specific HHRA accounts for bioavailability of soil As. Extensive research efforts have been directed toward development and application of in vitro gastrointestinal methods to predict relative bioavailable As (RBA As) across four continents. RBA As vs. IVBA As regression equations are used to predict RBA from IVBA. These methods are gaining regulatory acceptance for HHRA on contaminated sites. However, the ability of bioaccessibility methods to predict RBA As for contaminated sites and sources outside those used in developing the IVIVC regression equation is unknown. The objective of the current study is to evaluate the ability of several international bioaccessibility methods to predict RBA As for 12 contaminated sites. Arsenic bioaccessibility, determined using five in vitro methods, ranged from < 1 to >90% for 27 arsenic contaminated soils. A variety of linear regression methods have been used throughout literature to produce in vivo-in vitro correlations (IVIVC). Subsequently, soils were collected from 12 contaminated sites (i.e., locations). Bioaccessible As (IVBA As) was determined by several in vitro gastro(intestinal) methods including the SBRC, RBALP, USEPA Method 9200, OSU IVG, and the California Bioaccessibility method (CAB). Relative bioavailable (RBA) As was determined from dosing trials using juvenile swine. Regression equations from published IVIVC were used to predict RBA As from in vitro arsenic bioaccessibility. In general, the five IVBA bioaccessibility methods were predictive of RBA As for arsenic contaminated soils and solid wastes. Results from this study show IVBA methods provide reliable estimates of RBA As for use by contaminated site managers. However, several of the IVBA methods underpredicted RBA As when applied to specific sites. Despite As(V) adsorbed to mineral surfaces being a major component of most soils (>50%), soils ranged from ~20-80% in IVBA As and widely ranged in RBA As. Arsenic speciation alone is not predictive of IVBA or RBA As. However, As speciation is very important to provide information on IVBA or RBA As results. Supplemental site data (i.e., arsenic speciation) may be required for proper selection of methods by risk assessors to accurately predict RBA As and human exposure and gain acceptance by the regulatory community.

**665 Modeling the post-ingestion bioaccessibility of organic compounds sorbed to soils and house dusts**

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Ingestion of soils and house dusts with sorbed toxicants is an important pathway for children's exposure to many regulated organic chemicals. Therefore, it is important to understand the extent to which soil/dust sorbed toxicants mobilize (become bioaccessible) in the digestive tract. We used an in vitro, three-compartment system to model the bioaccessibility of the insecticide fipronil and 18 polychlorinated biphenyls (PCBs) from 37 soil and 37 house dust samples. For each sample. freely dissolved and total concentrations of fipronil and PCBs in the digestive fluid were measured and compared to concentrations remaining in the soil/dust sediments to calculate percent free ($BA_{frc}$) and total ($BA_{totc}$) bioaccessible fractions. Physicochemical soil and dust properties, and log octanol-water partition coefficients (log $K_{ow}$) of fipronil and PCBs were used to generate bioaccessibility models. In soil, mean $BA_{totc}$ were 89 ± 10% (fipronil) and 65 ± 16 % (PCBs). In house dust, mean $BA_{totc}$ were 58 ± 17% (fipronil) and 36 ± 14 % (PCBs). Most of the bioaccessible fipronil was freely dissolved whereas all measurable PCBs were bound to organic constituents or trapped in micelles. The significant bioaccessibility predictors were: Carbon (fipronil soil, $r^2 = 0.62$; fipronil dust, $r^2 = 0.30$; PCBs soil $r^2 = 0.65$) and carbon/clay (PCBs dust, $r^2 = 0.56$). Combined fipronil / PCB models of $BA_{totc}$ retained only carbon and log $K_{ow}$ for both soil ($r^2 = 0.78$) and dust ($r^2 = 0.65$). These results indicate that bioaccessibility can be modeled using properties of chemicals, soils, and house dusts.

**666 Utility of Urinary Metabolites in the Design and Execution of In Vivo Bioavailability Studies of PAHs**

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Recent and ongoing bioavailability testing of PAHs in soils and from complex mixtures like coal tar, coal tar pitch, and soot has included in vivo bioavailability testing and in vitro bioaccessibility testing. Despite its cost, in vivo bioavailability test has a key role to play whether used for a site risk assessment or used to validate in vitro tests. Animal tests of noncarcinogenic PAHs, such as pyrene and phenanthrene, and potentially carcinogenic PAHs, such as benzo(a)pyrene (BaP), have been aptly summarized in detail by Ruby et al. (2016). These tests have used different dosimetrics, including urinary metabolites, circulating blood levels, PAH adduct levels, etc. No one approach has proved to be superior. A recent study that received regulatory approval in 2017 and was summarized as a case study in the Interstate Technology Regulatory Council (2017) guidance document entitled *Bioavailability of Contaminants in Soil: Considerations for Human Health Risk Assessment*, was presented at this meeting last year (Forsberg et al., 2017). This mouse study of soil containing coal tar pitch from clay pigeon fragments at DOD shotgun range sites successfully used water soluble hydroxylated PAH metabolites in the urine as the dosimetric. Because of the low fraction of ingested dose excreted in the urine as 3-OH and 9-OH BaP (0.5% for soil groups and 2% for soil extract groups), one must plan studies that use urinary metabolites with care. This paper will discuss the potential difficulties, specifically the need for low detection limits, the instability of de-conjugated hydroxy metabolites in mouse urine, and the potential for confounding by fecal contamination.

**667 Nutrient availability effects on wheat plant root exudate composition and exudate effects on sorption of lamotrigine to soil**

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Irrigation with treated wastewater and soil amendment with biosolids are increasingly common practices in agriculture, and result in crop plant exposure to a variety of ionizable organic contaminants (IOCs). Plants may take up and accumulate IOCs, but our understanding of the variables that control IOC phytoavailability contains many gaps, including limited understanding of processes occurring at the soil-plant interface. The rhizosphere, the 2-3 mm of soil immediately surrounding roots, can have significantly different properties than bulk soil. Plants release ions and organic compounds from their roots to maximize nutrient availability. These root exudates can result in significant changes to the pH and chemistry of the rhizosphere. Root exudates may also alter sorption of pollutants, changing bioavailability and biodegradation potential. However, little is known about how changes in nutrient availability can alter the composition of root exudates or how exudates may impact sorption of IOCs, thereby impacting IOC availability to plants. We grew hydroponic wheat plants in a variety of nutrient solution compositions and collected their root exudates. We then analyzed the exudates using UV-Vis spectroscopy and Fourier Transform – Ion Cyclotron Resonance Mass Spectrometry, and compared exudates from differing growth conditions. We also measured sorption of the cationic anti-epileptic drug lamotrigine to three field soils with and without the presence of root exudates. Soil organic carbon was the major driver of lamotrigine sorption, and root exudates altered sorption most in soils with low organic carbon. Implications of our results on the bioavailability and accumulation of other IOCs will be discussed.

**668 Ecological risk assessment of lead in soils: A complete dataset to allow bioavailability correction for direct toxicity and secondary poisoning**

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Metal bioavailability and toxicity in soil can be strongly affected by soil physico-chemical properties and ageing. In a multi-year study, the lead industry has addressed these issues with a view to analyzing terrestrial lead risk assessment. For direct toxicity, the study included measuring toxicity of lead (Pb) for 6 endpoints (2 plants, 2 invertebrates and 2 microbial endpoints) in a set of 7 soils covering a representative range of soil properties (pH, organic matter content, texture and eCEC). The effect of ageing was quantified by studying toxicity of Pb after 3 different contamination conditions: i) freshly spiked with $PbCl_2$, ii) freshly spiked, leached and pH corrected in order to remove salinity and pH stress, and iii) freshly spiked and aged for 5 years. Variation in soil properties significantly explained variation in Pb toxicity for some endpoints (e.g. nitrification), but not for other endpoints (e.g. microbial respiration, *Folsomia candida* reproduction). Leaching and long-term aging of soils attenuated Pb toxicity significantly with a median factor of 4.7, confirming higher toxicity in freshly spiked soils compared to soils equilibrated under field conditions. In a recent study for secondary poisoning, the effects of soil properties on biota-soil accumulation factors (BSAF) have been characterized for earthworm species and the risk of Pb is assessed for the food-chain soil => earthworms => earthworm eating predators (birds and mammals). The BSAF values (dry wt) identified as reliable are variable (0.01 to 22.05; median 0.23; n= 248) and no soil properties except eCEC are significantly ($P< 0.01$) correlated with BSAF. Several bioavailability models, developed relating soil properties to Pb toxicity or BSAF, allow normalization of soil toxicity data to site-specific soil properties. Considering a relevant range (approximately 10th-90th percentile) of soil eCEC, safe levels of Pb for direct toxicity vary between 150 and 450 mg Pb/kg, while for secondary poisoning between 120 and 400 mg Pb/kg. A computer interface for user-friendly uses of the above models together with species sensitivity distribution (SSD) analyses will be demonstrated in the context of implementing bioavailability for predicting ecological risks of Pb in soil for a given site.

## Protecting Freshwaters from Salinization

**669 The Salton Sea: A real-life experiment of extreme freshwater salinization**

*W.L. Goodfellow, Exponent, Inc. / Ecological and Biological Sciences Practice; A.M. Deines, Exponent, Inc / Ecological and Biological Services Practice*

The Salton Sea is California's largest lake, located in the Imperial and Coachella valleys. The waterbody is 35 miles long, 15 miles wide, and 236 ft below sea level, with water flow into the lake from the New, Whitewater, and Alamo Rivers, as well as agriculture run-off, drainage systems, and creeks. The waterbody is a closed system with no outflow. In 1905, flooding occurred within the Colorado River system, resulting in a breach of an Imperial Valley dike. Over approximately two years, nearly the entire river filled the Salton Sink, creating the Salton Sea. The newly created lake quickly became a tourist and recreational area, with many commercial enterprises, hotels, and resorts developed along the shoreline. The lake also became a frequent stop along the Pacific Flyway, with over 400 species documented at the Salton Sea including substantial populations of the American white pelican, long-billed curlew, white-faced ibis, and the mountain plover. From the early 1900s until present, the salinity of the waterbody has increased from 3 to approximately 60 ppt, roughly at a rate of 1–2 ppt or more per year; recent water transfers from the Imperial Valley have further accelerated the increase in salinity concentrations. Fish stocked in the heyday of the Salton Sea resort community first included primarily freshwater species such as the threadfin shad, common carp, red shiner, channel catfish, white catfish, largemouth bass, and the desert pupfish. As salinity increased, marine species dominated including Orangemouth corvina, Bairdiella, and sargo. Tilapia though typically considered freshwater species escaped from local aquaculture and now dominate the fish community. Currently the fish population is largely composed of Mozambique tilapia and its hybrid, with populations thought to be located at the many seeps, drains, and creeks entering the lake. This presentation will discuss the impacts to the lake fish population, including the ability of the cichlid species to tolerate the rapidly increasing salinity concentrations. Additionally, this paper will present Salton Sea management and restoration plans recently proposed by the California Natural Resource Agencies.

**670 Using simulated background waters and site waters that represent field conditions to investigate salt toxicity**

*A. Farag, USGS / CERC / Jackson Field Research Station; D. Harper, USGS / Columbia Environmental Research Center*

While it is important to use traditional laboratory waters (e.g. moderately hard reconstituted) to limit variability and allow for inter-laboratory comparisons of salt toxicity results, applied science in field waters where salinization may be occurring is critical to advance salt toxicity research. Site waters and/or laboratory waters prepared to simulate field chemistry are not only useful for site specific applications, but provide additional ground truthing for salt models, proposed management actions, and restoration efforts. This laboratory previously conducted a series of experiments of to investigate $NaHCO_3^-$ in the Powder River Basin, Wyoming where treatment options appeared to reduce toxicity. Similar methods are now being applied to NaCl toxicity in waters of North Dakota and Montana. The current research focuses on investigating the toxicity of NaCl under conditions where oil and gas flowback spills and legacy contamination have occurred among productive agricultural lands and wetlands. Concentrations of Ca are generally elevated in these waters, which may ameliorate salt toxicity. Duckweed (*Lemna gibba*) and *Daphnia* (*D. magna*) were exposed to both a background water simulating the Prairie Pothole Region of North Dakota with NaCl added, and to site water collected from Goose Lake, Montana. EC50s were smaller (by > 500 mg $Cl^-$ mg/L) for both species in the site water compared to the simulated water. The site water generally had 500 mg Ca/L more than the simulated background water and results indicate that the additional Ca did not provide protection against toxicity. Though Ca is known to ameliorate toxicity of salts at lesser concentrations, the threshold of protection is below what exists in field situations of North Dakota and eastern Montana. This finding is applicable for any flowback or receiving waters with elevated hardness concentrations. For both simulated water and site water, duckweed was more sensitive with lesser toxicity thresholds than *Daphnia* . A chronic toxicity experiment was performed with duckweed and preliminary results suggest that the chronic EC50 is approximately 1/3 that of the acute EC50 for the simulated Prairie and Pothole water. This documented sensitivity of duckweed is especially important as managers investigate restoration goals and options. The resilience of aquatic and semi-aquatic plants to salts is of particular concern for wetland and riparian habitats.

**671 Transepithelial Potential as a Metric of Major Ion Toxicity in Fish: 2. Salt Mixture Studies**

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Salinization of fresh waters is increasing on a world-wide basis and there is an urgent need to develop tools for regulating major ions in freshwater ecosystems. The multi-ion toxicity model has been developed over the last several years to meet this need. The model is based on a mechanistic framework where disturbance in transepithelial potential (TEP) at the gills is the physiological endpoint used to predict toxicity. The TEP is the electrical potential gradient across the branchial epithelium, and is a key factor both affecting, and affected by, the movement of major ions between the normally dilute external environment and the normally concentrated blood plasma. Its disturbance is associated with a disruption of normal ionoregulatory homeostasis. Here we present initial experiments using the channel catfish (*Ictalurus punctatus*) on the effects of binary salt mixtures on fish TEP. This represents a first test of whether TEP can predict the toxicity of salt mixtures. The fish were exposed separately to three binary salt combinations $MgSO_4$:$Na_2SO_4$, NaCl:$CaCl_2$, $MgCl_2$:KCl. After evaluating an isobologram approach based on traditional practice for mixture toxicity testing, we instead tested the effect of varying one salt while holding the other salt at several constant concentrations, and then conducted the reciprocal experiment. Each experiment evaluated 10-12 treatments across a range of concentrations that encompassed the 96-h LC50. The binary salt mixtures were selected as representative mixtures that elicit additive, independent and mixed toxicity based on acute toxicity data. Acute changes in TEP were measured using a high impedance voltmeter, and indwelling peritoneal catheters connected to silver chloride electrodes via KCL-agar bridges. Several ways of analyzing the data will be explored. Overall the results of these binary mixture salt tests reinforce the mechanistic basis for the TEP model for predicting major ion toxicity and suggest further testing with a broader diversity of species and a broader range of salt combinations is warranted to further evaluate the model.

**672 Sulfate toxicity in the mayfly *N. triangulifer*: Pumping ions is energetically taxing**

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Elevated major ion concentrations in streams are commonly observed as a consequence of resource extraction, de-icing and other anthropogenic activities. Ecologists report biodiversity losses associated with increasing salinity, with mayflies typically being highly responsive to increases of different major ions. In this study, we evaluated the performance of the mayfly *Neocloeon triangulifer* reared for its entire larval phase in a gradient of sulfate concentrations ranging from 0.058 to 15.6 mM $SO_4$. Water from two oligotrophic streams was amended with sulfate as a blend of $CaSO_4$ and $MgSO_4$ at a 2.4:1 mass ratio of Ca:Mg. Survival (percent successful emergence to the subimago stage) was significantly reduced at the highest $SO_4$ concentration in both waters, while elevated $SO_4$ significantly delayed development in both waters. Final sub-adult body weights were generally consistent across treatments, except at the highest treatment concentration, where adult size and fitness were compromised. Subimagos that emerged from these exposures spanning 4 orders of magnitude of $SO_4$ showed only very modest increases in whole body S concentrations, suggesting that these animals are strict osmo-regulators. Sulfate uptake in *N. triangulifer* is concentration dependent and follows typical Michaelis-Menten kinetics. However, even at extremely high environmental $SO_4$ concentrations, SO4 uptake continues to increase. Thus the cost of maintaining relatively stable body S when water $SO_4$ concentrations are elevated imposes an energetic demand that is manifested primarily as reduced growth rates and associated developmental delays. We are in the process of identifying genes related to sulfate transport in *N. triangulifer* and examining how their expression changes as a function of environmental salinity.

**673 Transepithelial Potential as a Metric of Major Ion Toxicity in Fish: 1. Single Salt Studies**

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As salinization of fresh waters is increasing on a world-wide basis, there is an urgent requirement to develop regulatory tools, and for such tools there is a need for an integrative metric of toxicity. Building on a developed modeling paradigm, we explore a mechanistic approach where the metric is disturbance of the transepithelial potential (TEP) at the gills. The TEP is the electrical potential gradient across the branchial epithelium, and is a key factor both affecting, and affected by, the movement of major ions between the normally dilute external environment and the normally concentrated blood plasma. Its disturbance is associated with a disruption of normal ionoregulatory homeostasis.The premise is that major ion toxicity can be predicted as a function of changes in TEP, with a given change in TEP associated with toxicity regardless of the major ion concentrations and composition needed to elicit that change. This TEP approach evolves from theoretical predictions of the depolarizing effects of major waterborne ions, which can be made if their relative branchial permeabilities and their blood and plasma concentrations are known. These predictions rely on the Goldman-Hodgkin-Katz and Spangler equations, which are extensions of the well-known Nernst equation. Here we present proof-of-principle experiments on three freshwater fish species (fathead minnow *Pimephales promelas*, channel catfish *Ictalurus punctatus*, and bluegill sunfish *Lepomis macrochirus*). The fish were exposed separately to 8 different salts (KCl, NaCl, $MgCl_2$, $CaCl_2$, $K_2SO_4$, $Na_2SO_4$, $MgSO_4$, $CaSO_4$) across a range of concentrations that encompassed the 96-h LC50. Acute changes in TEP were measured using a high impedance voltmeter, and indwelling peritoneal catheters connected to silver chloride electrodes via KCl-agar bridges. Several ways of analyzing the data will be explored.

Overall the results of these single salt tests indicate that while there are species-specific differences in the magnitude of responses, within each species, there is remarkable consistency between several TEP metrics and toxicity. These results reinforce the mechanistic basis for the TEP model for predicting major ion toxicity, and have encouraged us to now test the approach using binary salt mixtures.

**674 Predicting the Aquatic Toxicity of Mixtures of the Major Ions: Technical Update – Chronic Exposures and Additional Test Species**

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Elevated concentrations of mixtures of seven major ions ($Ca^{2+}$, $Mg^{2+}$, $Na^{+}$, $K^{+}$, $Cl^{-}$, $SO_4^{2-}$, and $HCO_3^{-}$) have become a concern in some freshwater systems. The development of water quality criteria for these constituents has proven to be a challenging endeavor because toxicity of any specific ion has been found to be influenced by both the total concentration of ions in the mixture and, importantly, mixture composition. Use of an integrative measure such as TDS or conductivity to evaluate multi-ion toxicity (MIT), provides an indication of the total ion concentration, but fails to address mixture composition. Because of the aforementioned limitations of relating aquatic toxicity to specific ion concentrations or TDS/conductivity, we have pursued the problem from a different perspective – by relating toxicity to trans-epithelial potential (TEP), a physiological parameter that is computed based on blood or hemolymph and external major ion concentrations and calibrated model parameter values. This approach has been successfully applied to a limited number of relatively large acute toxicity datasets (*C. dubia*, *D. magna*, *P. promelas* and *N. triangulifer*) with widely varying ion compositions and, more recently, to much smaller, less robust datasets for species that would ultimately be included in the species sensitivity distribution (SSD). This presentation reviews how the approach has been extended to additional small acute and chronic effects datasets with multiple species for the purpose of testing the existing approach as well as expanding it to additional organisms and exposure durations.

**675 The accumulation and effects of persistent-energy related brine contamination on amphibian populations**

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The Williston Basin overlaps the North America's Prairie Pothole Region, one of the world's largest prairies and wetland ecosystems. Oil and gas production in the Williston Basin has increased rapidly over the last 20 to 30 years and secondary salinization from wastewaters (brines) co-produced during energy production have the potential to affect freshwaters. These wastewaters contain elevated concentrations of sodium, chloride and other metals which can alter water quality, persist in wetland sediments and negatively affect organisms Amphibians are especially sensitive to salinization with the increased possibility of additive and synergistic interactions among other pollutants. Few studies have examined potential interactions between brines and metals that readily accumulate in sediments and tissues. There has also been little research on the population- or community-level effects of salinization from oil-related waste products on aquatic species. We sampled 33 wetlands in Montana and North Dakota for 3 amphibian species, and used N-mixture models (estimate of animal abundance from spatially replicated data) to determine how abundance varied with evidence of brine contamination. Mixed-effects linear models were also used to measure the association between metals in sediments and tissues, to determine if accumulation varied by species and feeding behaviors (grazer, leopard and chorus frog vs. predator, salamander). Abundance of boreal chorus frog larvae declined most rapidly in response to elevated chloride (range: 0.04–17500 mg/L), followed by the northern leopard frog and barred tiger salamander. Concentrations of several metals were correlated with differences in feeding habits (e.g., omnivorous grazers vs. predators) rather than brine contamination. These results suggest that closer contact with the bottom sediment (i.e. grazers) could lead to greater ingestion of metal-laden soils and result in higher concentrations in tissues, particularly for those metals with higher assimilation efficiencies. Our results reveal contamination from saline wastewaters are associated with population- and community-level effects on wetland amphibians but it is not clear whether these effects are caused solely by brines or if other pollutants are also playing a role. A more robust understanding of these complex systems would help land managers prioritize conservation, remediation and restoration efforts in wetlands affected by historic energy production.

**676 Development of risked-based water quality guidelines for the protection against secondary freshwater salinisation in South African freshwater**

*P.K. Mensah, Rhodes University / Institute for Water Research*

Freshwater salinisation in South Africa is on an increasing trajectory with elevated levels of sulphate, sodium and chloride ions in many of the country's rivers. This phenomenon is mainly from secondary sources, including salinity due to agriculture, mining and domestic wastewater effluent. Thus, the aim of this study was to develop risked-based water quality guidelines (WQGs) for the protection of freshwater resources in South Africa. Short term and long term lethal exposure tests were conducted with indigenous South African aquatic organisms belonging to different taxonomic groupings. Ecotoxicological experiments were conducted by exposing different species of organisms to either NaCl (representing agriculture source), $Na_2SO_4$ (representing mining source) and wastewater effluents, in separate experiments. Static non-renewal experimental methods were employed for short-term lethal tests ($\leq$ 96 h), and static renewal for long-term lethal tests (> 96 h $\leq$ 21 d). The mortality results were used to estimate the lethal concentration (LCx) values. The LCx values from these experiments, those from salt database hosted by the Institute for Water Research (IWR) and those from literature search were used to develop risk-based WQGs using the species sensitivity distribution (SSD) approach. Different median protective concentrations (PC) with their lower and upper predicted intervals were estimated for different proportions of the population against exposure to NaCl, $Na_2SO_4$ and wastewater effluents. The median protective concentrations of 95 % of the population (PC95) values of distributions together with their lower and upper predicted intervals are used as the WQGs. The PC95 value is the mean guideline, while the lower-upper predicted values represent acceptable limits, i.e., acceptable or tolerable risks. Beyond these, risks are unacceptable.

## Advancements in Green Infrastructure Systems Through Life Cycle Assessments

**677 Sustainability Assessment of Green Infrastructure: The case of Urban Farms and Food Systems in US and India**

*A. Ramaswami, University of Minnesota / Humphrey School of Public Affairs*

Cities worldwide are seeing an emergence of policies and actions that are promoting green infrastructure and urban food system policies (e.g., UN Habitat; Milan Food Pact), with the latter often focusing on programs that promote urban farming. Often, many and diverse benefits are attributed to local green infrastructures and urban food systems – including environmental resource sustainability, health benefits, equity benefits, flood and heat resilience, and wellbeing. However, the magnitude of these benefits are poorly quantified and also often contested. Systems approaches are needed to assess these diverse sustainability impacts and co-benefits. This talk will present results of multi-objective sustainability assessments of urban food systems in cities in US and India, with implications for design of green infrastructure and urban farming.

**678 Environmental Impact Network Analysis of US Cold Food Supply Chain Post-Processing Storage**

*J. Burek, University of Arkansas / Chemical Engineering; D. Nutter, University of Arkansas / Department of Mechanical Engineering*

More research is necessary to understand the effects of an increasing global food cold supply chain. The role of distribution centers is to move or store food and other products. We felt compelled to assess the cold supply chain in the United States and identify which food had the highest and lowest storage impacts. The research provided national and state-level environmental impact assessment of cold storage in the United States. The multi-facility building network case study was based on the whole U.S. cold food supply freezer and cooler network. Distribution centers were characterized by zones within the building (cooler, freezer, and subfreezer), location, climate zones, typical meteorological year (TMY3), energy source for electricity production, and equipment. Furthermore, the research provided national-level environmental impact assessment of different food in cold storage. Finally, the research established procedures to calculate environmental impact of post-processing food cold storage in different states. In this research we used a life cycle assessment (LCA), and reported water, energy, and climate change impact of distribution centers based on geographic location. We used regional water consumption data and applied Available Water Remaining (AWARE) method, which provided assessment of water use based on building locations. Thus, this work also contributed to research in water-energy-food nexus. Some important findings included: 80% of energy use is attributed to dock, where food is loaded and unloaded. Previous research in food sustainability accounted only for average refrigeration of storage. Food storage added from 11% (dairy) to 78% (raw vegetables) to food global warming environmental impact. The models originating from this research are comprehensive process-based LCA models, which include accurate and reproducible building energy data. The models can be adapted for any other cold supply chain in the world, they allow performing scenario analysis including the indirect factors, such as change in technology and supply chain effects and external factors such as refrigerator choice and energy efficiency. The results will serve as benchmark to improve sustainability of distribution centers, and consequently food distribution.

**679 Valuing Organic Waste-to-Energy Systems**

*C.D. Scown, Lawrence Berkeley National Laboratory / Energy Analysis & Environmental Impacts; H. Breunig, Lawrence Berkeley National Laboratory; O. Kavvada, Lawrence Berkeley National Laboratory / Energy Analysis & Environmental Impacts*

Heterogeneous organic waste resources, particularly high-moisture wastes, are some of the most challenging feedstocks to derive value from because of the limited options for processing and the short time window during which such wastes can be stored. Municipal organic waste, for example, must be processed within 48 hours in California, once delivered to a facility. From an avoided impacts perspective, however, these wastes are also the most important to divert from landfills: they are fastest to degrade in landfills, meaning as much as half of fugitive methane emissions escape before the cells can be capped and gas capture system begins operating. Understanding the net greenhouse gas (GHG) and air pollutant emissions associated with the base case management of wet organic wastes, and the competing options for waste-to-energy systems is complex. Co-products, such as compost and biochar, introduce significant uncertainty as their role in soil carbon sequestration and avoided fertilizer application are highly variable by location and soil type. We present a detailed life-cycle assessment, with a primary focus on net GHG and air pollutant emission inventories, comparing both conventional and advanced strategies for utilizing wet organic wastes. Our study provides both a generalizable framework for analysis, accounting for co-products and ambiguity as to what the single functional unit should be, as well as two sets of case study results for actual facilities in California making use of food waste, manure, and green waste. We will present the GHG and air pollutant tradeoffs associated with different strategies, and highlight methodological choices that, while sound, may result in surprising or counterintuitive incentives. We will also highlight important data gaps requiring further research, and their resulting impact on uncertainty in the final results.

**680 Quantifying the potential environmental impact benefits of bio-derived polymers in bio-based composites for construction**

*S. Miller, University of Califorina Davis / Civil and Environmental Engineering*

The rising demand for construction materials is leading to significant consumption of natural resources and environmental impacts. The depletion of resources and the environmental concerns associated with currently available construction materials are pushing exploration of alternative materials. Among these alternative materials is the use of bio-derived material resources in the production of bio-based composites. Exploration of these composites has commonly included replacement of synthetic fibers with natural fibers and more recently, the replacement of fossil-derived polymers with bio-derived polymers, thus creating fully bio-based composites. Most research on these materials has focused on mechanical and material property assessment overlooking the relative benefits or drawbacks in environmental impacts from the use of bio-derived polymers as opposed to fossil-derived polymers. This presentation will examine greenhouse gas emissions and embodied energy, two critical areas of assessment in the comparison of bio-derived resources relative to fossil-derived resources, for several bio-based composites. Findings based on cradle-to-gate production of several wood fiber reinforced composites will be presented: three with fossil-derived polymer matrices and three with bio-derived polymer matrices. Results from this work suggest notable reductions in greenhouse gas emissions for use of bio-derived polymers; however, findings are not as pronounced in embodied energy for these composites. By combining environmental impact assessments with material properties from the literature, this work shows that through tailoring of fully bio-based composites environmentally desirable alternatives to conventional materials could be developed.

**681 Production and Use of Environmental Product Declarations for Pavements**

*J. Harvey, University of California, Davis / Civil and Env Engr; J. Meijer, The Right Environment; A. Butt, University of California, Davis / University of California Pavement Research Center; A. Mukherjee, Michigan Technological University / Civil & Environmental Engineering*

Similar to nutrition labels for processed foods, environmental product declarations (EPD) are published by product manufacturers to communicate the potential environmental impacts of a product or process. The International Standards Organization (ISO) has established processes that use life-cycle assessment (LCA) methods to declare the environmental impacts on product labels, called Type III EPDs. The pavement materials industries, the Federal Highway Administration, consultants and academics have been working together developing detailed guidance for producing EPDs for pavement materials, clarifying how they can be used, and technology transfer. The objectives of producing (materials producers), and requiring and using (agencies and other owners) EPDs are to: Make LCA-based information and additional information on the environmental aspects of products widely available and understandable. Assist purchasers and users to make informed comparisons between products. Encourage improvement of environmental performance in a fair and transparent manner. EPDs are produced following Product Category Rules (PCR), and in the U.S. most pavement material PCR operators are accredited certification bodies or industry organizations for a given product. In Europe, every country has a single national program operator for all products that is a non-profit organization. Harmonization of PCRs within the supply chain and between competing industries is an issue in the U.S. A comprehensive strategy is needed to review and reconcile

PCRs around standardized procedures. These issues have led to the discussion of whether there needs to be single PCR for the U.S., and creation of a governing consortium to manage the PCR. EPDs are being routinely produced and requested by agencies in several northern European countries. Although there are a number of benefits, there are also risks for industry in their production of EPDs and for agencies requesting them due to the lack of consistency across the PCRs under which EPDs are issued. Industries need agencies to strengthen the market by responding to the industry investment in the development of PCRs and EPDs, which means that agencies need to start requiring EPDs (several states have started). A general strategy and an approximate timeline for agencies based on the results of an FHWA supported workshop have been developed.

**682 Sustainability of building-scale net-zero water systems through an LCA lens**

*V. Hasik, University of Pittsburgh; H. Gardner, M. Bilec, University of Pittsburgh / Civil and Environmental Engineering*

Various green building rating systems advocate for buildings' implementation of rainwater harvesting and wastewater reuse to mitigate water scarcity and water quality issues; however, regional and technological factors may make implementation of some building-scale water systems less desirable on a life cycle basis, especially when considering direct on-site emissions and additional material and energy inputs. We used life cycle assessment to quantify the impacts of two state-of-the-art green buildings with net-zero water systems, considering their use of materials, energy, and direct on-site emissions. The two systems are similar in size but feature slight differences in their setup, with one being almost fully closed-system, while the other utilizing more traditional open-system setup. Other differences include different active treatment technologies, storage tank sizes and materials, and piping networks. Preliminary results of the material inputs reveal the effect of material selection for piping and storage tanks, enabling a 10% reduction in the global warming potential and a 45% reduction in fossil fuel depletion categories from material use. The global warming potential resulting from direct on-site emissions can be reduced by up to 90% by the use of aeration in septic tanks, but even then, they can account for up to 50% of the total global warming potential of the system. Energy used by the systems can also be significant when the systems' water circulation is not optimized, even though both systems use energy from solar panels. This study reveals the tradeoffs and benefits of buildings' utilization of on-site water management technologies and highlights best strategies for minimizing their environmental impact in similar green building projects.

**683 Using Multi-physics Computational Models and Life Cycle Assessment to Inform Financing Mechanisms for Green Infrastructure**

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A recent study indicated that between US$57 trillion to US$67 trillion in infrastructure spending is needed globally — approximately 5% of gross world product annually from now until 2030. This amount, which reflects a 60% increase over historical spending levels, is needed to build technologically-advanced, more sustainable infrastructure in developing countries, and upgrade to technologically-advanced, more sustainable infrastructure in developed countries. For many municipalities and governments, however, the systems and technologies that enable this new generation of infrastructure are out of reach because governments lack funding or justification to plan systems that are more expensive to design, more expensive to build, and (in some cases) more expensive to operate. This research links the sensing of major infrastructure systems (i.e., tunnels, bridges), the multi-physics modeling of these systems, and the optimization of their management under future uncertainty to new "science-based" credit-risk models used by global financial institutions. For publicly and privately financed sustainable infrastructure, these models enhance financial risk management methods by assessing infrastructure cost volatility more accurately over the infrastructure lifecycle for a portfolio of systems. This cost volatility of infrastructure can then be juxtaposed against revenue and overall fiscal volatility within the financial sector. These models of volatility are built from the "bottom up" using a probabilistic model of future infrastructure cash flows that are directly informed by sensing of infrastructure performance, multi-physics modeling, and optimization algorithms. This research fundamentally links the design, construction, and life cycle management of major infrastructure with the global financial system and groups of investors that finance its development and operation. To demonstrate the framework, a simple reinforced concrete infastruture element is modeled in this presentatino using a newly developed multi-physics software suite and commercially available LCA software to inform a new financial assessment.

## Assessing Contaminant Effects in Ecosystems with Multiple Stressors with a Focus on the California Bay-Delta

**685 The science underlying management of chemical contaminants and nutrients in the Sacramento-San Joaquin Delta**

*T. Collier, E.A. Canuel, V.H. Resh, Delta Independent Science Board*

As part of its charge to review science activities that support adaptive management in the Sacramento-San Joaquin Delta, the Delta Independent Science Board (DISB) is reviewing the scientific basis for assessing and managing water quality in the Delta. The first phase of this effort has focused on chemical contaminants and nutrients, which pose multiple stresses on the Delta ecosystem, and on human uses of the Delta. Classes of chemical contaminants covered included pesticides, mercury and selenium, and contaminants of emerging concern. To assess the state of water quality science in the Delta, information was gathered from: a literature review of recent publications on the topic of water quality; responses to a questionnaire distributed to several agencies; in-person interviews with individuals involved in different aspects of water quality; and public comments. We also invited presentations to the DISB, and members of the DISB attended meetings of several working groups involved with water quality in the Delta. Our main findings are: It is not clear whether water quality data being collected are sufficient to support optimal management decisions and policies, and how water quality data are being used in management decisions. This seems particularly true for ecosystem management decisions. Adaptive management, as outlined by the Delta Plan and the DISB in previous documents, is rarely built into water quality programs. Water quality too rarely enters into discussions about water supply and reliability. Water conveyance and storage can influence Delta water quality by affecting where, when, how, and how much freshwater is diverted. Although several entities in the Delta fund research and monitoring activities aimed at protecting water quality in the Delta, these resources tend to support specific compliance needs. We also made ten general findings, and provide recommendations for each finding--this presentation will summarize our findings and recommendations. We will also discuss how chemical contaminants and nutrients can combine with other stressors in the Delta to contribute to organismal- and population-level effects.

**686 Multiple stressor impacts from the San Francisco Estuary on the reproductive health of the threatened Delta smelt, *Hypomesus transpacificus***

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The physical habitat, health, and nutritional indices of Delta Smelt, *Hypomesus transpacificus,* were studied in relation to reproductive status among regions of the Suisun/Delta. Adult Delta smelt were collected from routine surveys during the spawning period by the California Department of Fish and Wildlife from 2012 to 2015. Health indices of fish were evaluated by using morphometric and liver indices. Nutritional indices consisted of RNA/DNA and liver glycogen. Reproductive indices of GSI, oocyte stage, clutch size, oocyte size, egg weight, and estrogen level were collected for most of the year classes. Spatial and temporal factors were evaluated as well as migratory status on the reproductive indices. Indices of HSI and liver lesion severity correlated with reproductive indices suggesting that hepatotoxins may have impacted the reproductive status. Nutritional index of Glycogen was found to positively correlate with reproductive indices suggesting that good nutritional status was important for improved reproductive status. Reproductive status of Delta smelt in the brackish open water bays of their distribution was poorer than Delta smelt found in the shallows of brackish marshes and freshwater wetlands, suggesting that these areas are important for spawning.

**687 Combined exposure to EDCs and elevated temperature influences development, reproduction and gene expression across generations in an estuarine fish**

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Understanding anthropogenic impacts, such as climate change and pollution, on aquatic ecosystems is critical for preserving biodiversity and maintaining water quality. The pyrethroid pesticide bifenthrin is a known endocrine disrupting compound (EDC) that enters watersheds through urban and agricultural runoff. Ethinylestradiol (EE2) is a common pharmaceutical that enters watersheds via wastewater effluent. Little is known about how elevated temperatures associated with climate change may affect the estrogenic activity of bifenthrin and EE2, particularly in species that exhibit temperature-dependent sex determination (TSD), such as *Menidia beryllina,* which is commonly found in the California Bay-Delta estuary. This study investigated the effects of temperature and bifenthrin exposure on development, reproductive output and gene expression in *M. beryllina* across multiple generations. Fish in the parental generation were exposed to environmentally relevant concentrations of bifenthrin and EE2, as well controls at 22°C and 28°C for 14 days prior to spawning. Embryos in the F1 generation were exposed to EDCs as larvae (until 21 dph) and then reared to adulthood (8 months) in clean water at experimental temperatures. Sex ratios of the F1 generation were influenced by both elevated temperature and EDCs, resulting in alteration of adaptive TSD. In all F2 treatments, elevated temperature resulted in fewer viable offspring, with effects of individual EDCs affecting offspring production at lower temperatures. Fish exposed to bifenthrin during development exhibited developmental deformities. Changes in gene expression were observed in two generations larval fish following EDC exposure at 28°C, including those exposed indirectly as germ cells during early life-stage exposure of parents, suggesting that these effects may be transferrable across generations. These results illustrate the importance for testing the effects of multiple stressors concurrently, as effects can last through multiple generations. Findings from this study will be useful in determining how EDCs will impact organisms and community structure in the face of global climate change.

**688 The Effects of Temperature and Bifenthrin on the Behavior and Neuroendocrinology of Chinook Salmon (Oncorhynchus tshawytscha)**

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Coastal California has been experiencing the effects of chemical and non-chemical anthropological stressors through increasing surface water temperatures and rainstorm runoff events of pyrethroid pesticides washing into waterways, such as the San Francisco Bay-Delta. Salmonid populations in the Bay-Delta have been dramatically declining in recent decades, and the Bay-Delta is a delicate ecosystem rearing ground for juvenile anadromous salmonids. Therefore, the aim of this study is to investigate the effects of the pyrethroid pesticide, bifenthrin, and increasing water temperatures on the neuroendocrine response of Chinook (*Oncorhynchus tshawytscha*) salmon parr. Parr were reared at $11^0$C, $16.4^0$C, or $19^0$C for 14 days and, in the final 96 hours of rearing, parr were exposed to 0, 0.15, or 1.5 µg/L bifenthrin. A predatory avoidance Y-Maze behavioral assay was conducted immediately following exposures using taurocholic acid as a predatory odorant. Parr were presented a choice of clean or odorant zones, and locomotive behavior was video recorded. Plasma was harvested for thyroid hormone (T3 and T4), estradiol, and testosterone analysis using ELISAs. Brain and olfactory bulbs were collected for qPCR to analyze the relative expression of hormone and dopamine receptor genes. While no significant changes were observed in brain transcripts or hormone concentrations at any bifenthrin concentration or rearing temperature, there was a significant decrease in survival in parr reared at $19^0$C alone. Moreover, parr reared at $11^0$C and exposed to 1.5 µg/L bifenthrin spent significantly less time avoiding the predatory odorant compared to vehicle controls reared at $11^0$C . These results suggest that temperature may be impacting behavior at this juvenile stage, which may have population-level impacts on Bay-Delta salmonid populations.

**689 Multi-stressor effects of ultraviolet light, temperature, and salinity on oil toxicity in estuarine species**

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The cumulative and interactive stressors of chemical contaminants and environmental factors are especially relevant in estuaries where tidal fluctuations cause wide variability in salinity and temperature. Changes in depth also affect ultraviolet (UV) light penetration, which is an important modifying factor for polycyclic aromatic hydrocarbon (PAH) toxicity. The introduction of oil into estuarine systems may have different levels of effect depending on the tidal stage and time of year. Characterizing the interactions of multiple stressors on oil toxicity will improve prediction of environmental impacts under various spill scenarios. This study examined how toxicity of unweathered Louisiana Sweet Crude (LSC) oil was altered by temperature, salinity, and UV light. Several estuarine species representing different trophic levels and habitats were evaluated, including the sheepshead minnow, *Cyprinodon variegatus*, the grass shrimp, *Palaemonetes pugio*, and the mud snail, *Ilyanassa obsoleta*. These species are capable of surviving a wide range of environmental conditions, however, they were more sensitive to oil exposure when combined with abiotic stressors. UV light increased the toxicity of LSC oil in all species tested. LSC oil toxicity was also greater under elevated temperature conditions. Effects of salinity on LSC oil toxicity varied among species tested. Complex interactions occurred when more than one abiotic stressor (e.g. UV light and increased temperature) were combined with LSC oil.

**690 Contrasting multi-stressor effects on biological communities between urban and agricultural streams**

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Multiple physical and chemical stressors can simultaneously affect the biological condition of streams. Understanding these stressor-response relations can be challenging due, in part, to natural variation, spatial and temporal variation in stressors, and interactions among multiple stressors. To address this complexity, the U.S. Geological Survey sampled 98 streams in the Midwest Corn Belt and 75 streams in the Southeast Piedmont of the United States, representing agricultural and urban disturbance gradients, respectively. Water and sediment chemistry, streamflow, and temperature were characterized for up to 14 weeks prior to ecological surveys of habitat and algal, invertebrate, and fish communities in each stream. Boosted regression tree (BRT) models were developed for biological metrics based on landscape variables (GIS models) and based on in-stream stressors (stressor models). Generally, physical habitat characteristics were more important in the agricultural gradient models; 56% of the variables selected in stressor models in the Midwest Corn Belt were habitat-based, and 12% were contaminants-based, primarily pesticides. In contrast, contaminants and flow alteration dominated the urban gradient models; 43% of the stressor variables in the Southeast Piedmont models were contaminants, and 23% were habitat. Additional stressor variables contributing significantly to both agricultural and urban models were nutrients, dissolved oxygen, and temperature. GIS models, primarily based on land-use variables, performed well in both regions. The stressor models improve our understanding of which specific stressors and stressor combinations have the strongest influence on biological condition whereas the GIS models can be used for prediction to unsampled streams.

**691 Mixtures of Fungicides and Insecticides Occur Frequently in Central California Coastal Streams with Urban and Agricultural Land Uses**

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Pesticide mixtures are prevalent in U.S. streams. To evaluate their significance as stressors on aquatic biota, it is important to measure a variety of current-use pesticides at concentrations low enough to assess potential aquatic toxicity. In 2017, the U.S. Geological Survey conducted a multistressor study that measured pesticides in 85 small streams in the central California coast region over a 6-week period. Concentrations of 253 current-use pesticides and pesticide degradates were determined by liquid chromatography tandem mass spectrometry (LC-MS/MS) in filtered stream-water samples collected weekly. There were 107 pesticide compounds detected at least once at concentrations from 1 to 10,000 ng/L, and 27 pesticide compounds were detected in at least 10% of samples. Eleven pesticide compounds were detected in more than 40% of water samples: the insecticides chlorantraniliprole, methoxyfenozide, dinotefuran, imidacloprid, clothianidin and thiamethoxam; the fungicides boscalid, carbendazim, azoxystrobin, and myclobutanil; and the herbicide prometon. Pesticide mixtures tended to be most complex at sites in mixed land-use watersheds, where as many as 17 fungicides and 22 insecticides were detected per sample. Potential for adverse effects on aquatic life were assessed using USEPA Office of Pesticide Programs (OPP) aquatic-life benchmarks and the Pesticide Toxicity Index (PTI), although no benchmarks were available for about one-third of the detected pesticide compounds. OPP acute-invertebrate benchmarks were exceeded in one or more samples at five sites by the insecticides imidacloprid, methomyl, diflubenzuron or (cumulatively) the organophosphates. OPP chronic-invertebrate benchmarks were exceeded at 20 sites by 21-day average concentrations of one or more insecticides, including fipronil, imidacloprid, and clothianidin. The fungicide pyraclostrobin exceeded acute benchmarks for fish and nonvascular plants at one site. At seven sites, the PTI predicted that pesticide mixtures would be acutely toxic to invertebrates. The study results show the utility of using a broad-spectrum, sensitive method that targets a large number of current-use pesticides to characterize pesticide mixtures and to estimate potential aquatic toxicity. Insecticides were predicted to be important stressors to aquatic organisms in the California streams sampled.

**692 Managing risk for the California Bay-Delta, the multiple sources-stressors-habitats-endpoints paradigm and implementation of adaptive management**

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In reality, the status of an environment is due to the presences of multiple sources of multiple stressors affecting a series of spatially located habitats affecting a number of endpoints, many of which contribute to a series of ecosystem services. This framework can be abbreviated as multiple sources, stressors, habitats and endpoints (MSSHE). However, the mental models of many scientists, regulators, policy makers and stakeholders are limited to narrow questions, limiting the answers and ignoring context. No doubt some of this limitation is due to the legislative process giving rise to NEPA, Clean Water and Clean Air Acts, FIFRA, TSCA, RCRA and CERCLA and the various State and international equivalents. This way of conducting business is like mandating that only a compass and not a GPS unit be used for navigation. Similarly, the goal of returning a system to an historical unimpacted by human state is equally fantastic. Humans alter their habitats and the structure of the resident community, whether indigenous or recent migrant. Since the late 1990s and early 2000s it was demonstrated that it was possible to estimate risk to multiple endpoints due to multiple stressors using the relative risk model (RRM) and other approaches. Beginning in the mid-2000s Bayesian networks have been used to estimate risk to multiple endpoints due to multiple stressors and now the RRM has been reformulated as a Bayesian network. It has been demonstrated by multiple groups that probabilistic ecological risk assessments that are regional in scale can be performed. Molecular interactions, such as those described by adverse outcome pathways can be integrated with environmental factors and management alternatives. Recently it was demonstrated that the Bayesian network-RRM can be a critical part of an adaptive management program. Instead of primarily technical issues the glacial pace of adoption of the MSSHE approach appears to be 1) cages of regulatory bars based on the risk assessment tools of the 1990s, 2) educational systems that produces graduates that lack fundamental understandings of causality, analytical tools and ecological interactions, and 3) jersey barriers blocking communication between stakeholders, policy makers and scientists on what is now possible.

## Current-Use Pesticides: Exposure and Effects on Non-Target Organisms and Ecosystems – Part 2

**693 Neonicotinoid concentrations in surface waters of North America – Evaluation of potential chronic concentrations**

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The presence of neonicotinoids in surface waters of North America has been documented in several publications, but these data tend to lack sufficient sampling frequency to evaluate the potential dissipation of residues in these waters. Neonicotinoids are known to dissipate in water through photolytic processes, but this is often not considered when evaluating concentrations from such studies. Therefore, maximum (acute) concentrations are often compared to chronic toxicity endpoints. The temporal aspects of aquatic concentrations are critical to understanding potential effects on aquatic organisms, specifically aquatic invertebrates in the case of neonicotinoids. Results from ongoing, neonicotinoid-targeted, field-scale monitoring studies in prairie wetlands will be described, along with an evaluation of the temporal and spatial aspects of the observed water concentrations.

**694 Large-scale risk assessment of pesticides in Canadian Prairie wetlands**

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Wetlands are essential freshwater ecosystems in the Canadian Prairie Pothole Region (PPR), but they are frequently contaminated with pesticides, as a result of intensive agricultural activities in the region. We conducted a large-scale monitoring program examining the distribution and occurrence of a large suite of 169 current-use pesticides (insecticides, herbicides and fungicides) including groups of particular interest, such as neonicotinoid insecticides, and assessed the risk that they pose to wetland aquatic ecosystems. To prioritize sampling areas, we used a pesticide distribution model developed from land use imagery, and recent pesticide use survey data. Approximately 160 wetlands spanning the three Canadian Prairie Provinces were monitored for pesticides. These wetlands were predominantly semi-permanent ponds located in croplands. Water samples were collected pre-seeding (April/May) and post-seeding (June/July) in 2017-2018 with one additional monitoring campaign in the fall of 2017. Pesticide concentrations in water samples were compared against relevant ecological thresholds to determine the ecological risk to wetland health. In 2017, 63% of the wetlands had at least one pesticide detected. In total, 22 pesticides were detected, mainly in spring and summer. Some of the most frequently detected pesticides included neonicotinoid insecticides (i.e., thiamethoxam[18%]), the fungicide metalaxyl (15%), and the herbicide MCPA (50%). Concentrations of clothianidin and thiamethoxam exceeded ecological relevant thresholds at 45% and 40% of the sites, respectively. Mixtures of two or more pesticides were observed in 74% of sites (range 2-8 compounds per site). These mixtures occurred more frequently in the southeastern part of the PPR. The spatial distribution of pesticides varied by land use, with high concentrations detected in wetlands surrounded by canola and wheat crops. Overall, these results will help prioritize areas for further study, and establish the most relevant agrochemicals by mass and toxicity in the Canadian Prairies.

**695 Assessing Avian Exposure to Current-Use Pesticides**

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Many pesticides are currently applied in both agricultural and urban settings, resulting in chemical residues in various environmental compartments. Non-target organisms are exposed to a mixture of pesticides through direct and indirect pathways, potentially resulting in adverse effects. Pesticide exposure of biota is often modeled using concentrations detected in external media (water, sediment, soil, air). However, direct measurements of pesticides and metabolites in biological tissues may better characterize exposure and potential toxicological endpoints. Herein, we report on three field and laboratory studies to evaluate exposure of birds to current-use pesticides. Shorebirds breeding in the Pacific Northwest, US are exposed to pesticides from surrounding agricultural fields and water bodies. Analyses revealed that shorebird eggs contained five fungicides and two insecticides with concentrations varying by site and species, demonstrating that birds accumulate pesticides and transfer residues to their eggs. Next, a kinetic study was conducted with Japanese Quail (a model seed-eating avian species) dosed with treated wheats seeds containing one insecticide (imidacloprid) and three fungicides (metalaxyl, tebuconazole, fludioxonil). Pesticide and metabolite residues were quantified in brain, liver, kidney, muscle, plasma, and feces to determine their distribution and fate. Imidacloprid, tebuconazole, and metalaxyl were detected in all tissue samples and metabolites were observed for imidacloprid and tebuconazole. Imidacloprid metabolites were much more prevalent than the parent compound in all tissues except brain, highlighting the importance of screening for both pesticides and their metabolites in biological samples to holistically examine exposure. We then returned to the field to estimate pesticide exposure of Tree swallows (insectivores) from an agricultural region in central Saskatchewan, Canada. Non-lethal sampling techniques were used to collect food boluses from nestlings and blood from adults. Analyses of over 150 pesticides, metabolites, and degradates were conducted to determine environmental and dietary exposure. Analytical methods must continually evolve to monitor new inputs and transformation products in different biological matrices. This approach permits toxicologists and ecologists to link tissue concentrations of pesticides and metabolites to potentially adverse effects.

**696 Risks of insecticide exposure on monarch butterfly (Danaus plexippus) at the patch and landscape scales**

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North American monarch butterfly populations have declined by over 80% in the last two decades, and its potential listing as a threatened species is currently under review. To increase the eastern monarch population, milkweed (*Asclepias* species), the host plant of monarch larvae, need to be established in the agricultural landscapes of the U.S. Midwestern states. As insecticides are often used in these landscapes, it is important to assess the risks of insecticide exposure on monarch larvae at the patch and landscape scale. Larvae could be exposed to spray drift from foliar applications through the cuticle or by consuming milkweed leaves containing insecticide residues. Previously, we reported on risks from cuticular exposure; this presentation reports risks from oral exposure. Oral toxicity studies were undertaken with beta-cyfluthrin (a pyrethroid), chlorpyrifos (an organophosphate) chlorantraniliprole (an anthranilic diamide) and imidacloprid, clothianidin and thiamethoxam (neonicotinoids). Dose-response curves generated from acute studies were compared with exposure estimated from a spray drift model (AgDRIFT). For aerial applications, predicted percent mortality for 3rd instars ranged from 99% to 89% and 93% to 16% at the edge of field and 100 feet downwind from treated fields, respectively. Predicted mortality rates were higher for chlorantraniliprole and chlorpyrifos and lower for thiamethoxam. Chronic oral toxicity studies are in progress to assess risks to larvae from dietary exposure to seed treatment insecticides absorbed by milkweed downslope of corn and soybean fields. The estimated larval survival and developmental rates derived from field scale assessments are being incorporated into a projection model to predict the number of adult monarchs recruited at the landscape level. This research will help inform the conservation costs and benefits of establishing habitat in areas potentially exposed to insecticides.

**697 Decline of aquatic insect abundance and diversity in Santa Barbara creeks potentially related to increase in use of neonicotinoids and fipronil**

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We analyzed annual data from nine streams to determine if sensitive benthic macroinvertebrate (BMI) metrics changed from 2000 to 2012 in Santa Barbara creeks during a time of rapid increase in the use of imidacloprid, other neonicotinoids, and fipronil. This study was motivated by hydrochemical monitoring of stream and estuarine sites in Santa Barbara, which revealed frequent exceedances of biological chronic toxicity thresholds for imidacloprid and fipronil for weeks after storm events. We focused on temporal changes in mayfly (Emphemoptera) abundance given the reported high sensitivity of this group to chronic, low levels of imidacloprid, as well as changes in total invertebrate abundance owing to documented general declines in winged insect abundance. After controlling for the effects of land use and annual rainfall using multiple regression analysis, we found a significant decline from 2000 to 2012 for several invertebrate metrics (standardized regression coefficient, *p* value), including log[invertebrate abundance] (-0.35, < 0.0001), log[non-Baetid mayfly abundance] (-0.24, < 0.0001), insect taxonomic richness (-0.19, 0.001), logit[proportion shredders] (-0.13, 0.05), and EPT richness (-0.12, 0.03). These declines were detected in streams across the land use spectrum (moderately to heavily affected by urban and/or agricultural development, as well as reference locations). Invertebrate metrics were not related to regional mean air temperature (previous year or previous spring). Metric values at references sites may have declined owing to pollutant impacts (e.g., broad scale pesticide dispersion, lack of adult invertebrate dispersal from impacted sites, pesticides leached from dogs along hiking trails) or to direct (temperature, but see above) and indirect (dissolved oxygen) factors related to climatic cycles and trends. These results do not provide a direct connection between systemic pesticides and invertebrate metrics or discount other long-term drivers of invertebrate change; however, to date, the only pattern we have found suggests an association between invertebrate declines and increasing neonicotinoid use, emphasizing the importance of expanded research on this topic.

**698 Impact of neonic use in intensive agriculture on aquatic invertebrate community health: Analysis of long-term invertebrate biomonitoring database**

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Neonicotinoids (neonics) are considered a major innovation in agriculture with their ability to manage destructive agricultural pests at lower rates with more favorable ecological profiles than many alternatives. However, in recent years, concerns with regards to the ecological safety of neonics to aquatic invertebrates have increased. Iowa has the highest estimated use of neonics in the USA, based on total active ingredient applied, and maintains a statewide aquatic invertebrate monitoring network (more than 900 sampling locations) with extensive historical data, making it an ideal case study to retrospectively evaluate the potential impacts of neonic use on aquatic invertebrate community health function. We used aquatic invertebrate monitoring data obtained from the Iowa monitoring program to investigate whether aquatic invertebrate health and system function are associated with the intensity of neonic use in the watershed, and if increasing neonic use over time results in decreased aquatic invertebrate community health or impaired function. For each aquatic invertebrate sampling location, the contributing watershed area was calculated as well as the annual percent cropped area (PCA) for crops with potential neonic use. We found no correlation of watershed area or PCA with an index of aquatic invertebrate health or system function. Further, our analyses demonstrates the overall health of Iowa's aquatic invertebrate communities has been preserved despite the increased extent and geographic footprint of neonic use in Iowa associated with registration of new uses and adoption of the technology. These findings provide real world, large scale evidence, that current registered uses of neonicotinoid insecticides are safe to aquatic invertebrate communities.

**699 Seasonal alterations in the effects of neonicotinoid insecticide mixture toxicity on the prevalence of marine crustaceans in estuarine areas**

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In the present study, we evaluated the impact of neonicotinoid insecticides on the estuarine decapod crustaceans kuruma prawn (*Penaeus japonicus*) and sand shrimp (*Crangon uritai*), using a mixture toxicity model. Initially, 96 h toxicity studies were performed in the laboratory using the crustaceans with a body length of 12–17 mm. Kuruma prawn was observed to have a higher sensitivity to insecticides than that of the sand shrimp. The 96 h EC50 values ranged from 14 µg/L (clothianidin; Clo) to 943 µg/L (thiamethoxam) for kuruma prawn and from 255 µg/L (Clo) to 5,181 µg/L (nitenpyram) for sand shrimp. In subsequent field studies, 145 ambient water samples were collected at four estuarine sampling sites in the Seto Inland Sea of Japan, from June to December in the years 2015–2017. Crustaceans were harvested each month at one of the sampling points. The presence of the crustaceans was noted, and the occurrence of insecticides in estuary waters was measured. Five out of the seven neonicotinoid insecticides were identified in the estuarine water samples. The main insecticides detected were dinotefuran (Din), imidacloprid (Imi), and Clo at the following concentrations (with detection frequencies): < 0.001–0.74 µg/L (99%), < 0.004–0.18 µg/L (34%), < 0.002–0.07 µg/L (31%) respectively, when the concentrations were adjusted to salinity 20. Moreover, the occurrence of the insecticide showed temporal trends, which were correlated with their use in rice cultivation. Din and Clo were detected at a higher frequency in June and September, whereas Imi was detected at a higher frequency in June. Sand shrimps were found to be more abundant in early summer (June and July), whereas kuruma prawns were more abundant in autumn (September). Data on toxicity and occurrence of insecticides in the environment was analyzed using the response additive mixture model to calculate the maximum multi-substance potentially affected fractions (msPAFs), which were 0.8% (June) and 0.3% (September) for kuruma prawn, and 0.03% (July) and 0.002% (September) for sand shrimp. Based on the temporal appearance of the crustaceans, msPAFs in estuaries were maximized at 0.3% and 0.03% for kuruma prawn and sand shrimp, respectively. The present study demonstrates that neonicotinoid insecticides can pose a seasonal risk to estuarine crustaceans, and highlights the need to consider whether ecological risk periods coincide with crustacean emergence in estuarine areas.

**700 Relative abundance trends of bird populations in high intensity croplands in the Central US**

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Declining bird populations across the US have been noted in a number of studies. Although multiple explanations have been proposed as causes of these declines, agricultural intensification has often been suggested as a significant driver of bird population dynamics. We examined this relationship by comparing bird count data from the Breeding Bird Survey collected from 1995-2016 across 13 states in the central US, to corresponding categorical changes in land cover within a 2 km radius of each survey transect using spatially explicit USDA-NASS Cropland Data Layer. This approach allowed us to compare the slopes of counts for 31 species of birds between grassland- and cropland-dominated landscapes, and against increasing levels of cropland (all types combined) and pooled corn/soybean land cover types. Nearly all birds demonstrated significant responses to land cover changes. In all cases, the number of species exhibiting positive or negative responses was comparable and median differences in percent change per year ranged from -0.5 to 0.5 percent. Species that responded either positively or negatively did not appear to fall into any particular foraging guild. If changes in agricultural practices are a major cause of declines, we would expect to see it across the spatial scale studied and across the majority of species. While these results do not rule out that potential agricultural impacts, such as toxicity resultant from pesticide exposure, may have species specific or localized impacts, a variety of factors related to habitat are likely the most significant contributor overall. Given these results over a large spatial scale basis (multi-state) and across numerous bird species, there is not a broad general trend of greater decline in crop intensive areas.

# Chemical, Biological and Instrumental Methods for Detecting Harmful Algae Blooms and Their Natural Toxins

### MP001 Conceptual Site Models for Evaluating Microcystin Fate and Effects Following Upland Placement of Dredged Material from HAB-Impacted Waters

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Beneficial use of material dredged from navigable waterways—including placement for ecosystem restoration or brownfield redevelopment—is an increasingly favored dredged material management method. However, dredged material impacted by harmful algal blooms (HABs) potentially introduces algal toxins (e.g., microcystins) to these beneficial use environments, with subsequent new exposure considerations. A better understanding of the fate and effects of microcystin toxins contained within or sorbed to sediments subsequently dredged from a water body is needed in order to avoid adverse impacts. Recent sampling and analysis of dredged material indicates possible persistence of microcystin toxins in the upland environment one year after dredged material was removed from Lake Erie and placed upland. Conceptual site models were developed to discern the transfer, transformation, and exposure potentials for microcystins via upland or nearshore placement of dredged material. Additionally, critical data gaps that are limiting the understanding of fate and effects of microcystins in upland environments were identified. Several key research areas were identified, including persistence, degradation processes, and bioavailability of microcystin toxins in dredged material with varying characteristics (such as grain size and organic carbon content) and process methods (such as discharge and dewatering). Significant information gaps also exist for characterizing human health risks associated with exposure to microcystin toxins in the upland environment, with current research generally limited to exposure through drinking water and uptake into agricultural crops via impacted irrigation water. Although similar research is being conducted for land application of water treatment residuals that contain microcystin toxins removed from drinking water, dredged material and water treatment residuals have different chemical, physical, and biological characteristics which may affect microcystin fate and effects from these exposure scenarios.

### MP002 Prevalence of sediment-bound microcystin-LR detections in California's Stream Pollution Trends (SPoT) Program

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Microcystins are an hepatotoxic class of cyanotoxins which affect diverse taxa. Microcystins may persist in the environment for weeks to months, posing a threat to drinking water and aquatic habitats. While some variants display a high affinity to bind to sediment, most monitoring efforts have focused exclusively on water samples. California's Stream Pollution Trends (SPoT) Monitoring Program assesses long-term trends in sediment contaminants and toxicity. In collaboration with California State University Monterey Bay (CSUMB), an optimized method for extraction of MC from sediments was developed to analyze SPoT sediments. Sediment-bound microcystin was extracted and purified using a modified sodium pyrophosphate and solid-phase extraction column method and quantified with enzyme-linked immunosorbent assay (ELISA). Annual samples from fifty sites statewide were analyzed between 2014 and 2016. Microcystin-LR, one of the most common and toxic variants, was detected in 57% of samples in 2014, 33% in 2015 and 56% in 2016, at median concentrations of 0.38 ng/g, 0.176 ng/g, and 0.183 ng/g, respectively. Ten samples were extracted and analyzed separately for external method validation using both ELISA and LC-MS. Relative percent differences in the ELISA ranged from 16% to 174%. Individually, four sites showed significantly increasing concentrations (8%), but no sites showed significant decreases. To assess seasonal trends, four sites were sampled and analyzed quarterly (n=12 per site), but no significant trends in detections or concentrations were observed. There were no significant relationships between microcystin concentrations and proximate land use, measured toxicity and contaminants, or conventional field water quality measurements. Bioaccumulation of microcystin was confirmed with a 7-day laboratory exposure of the midge *Chironomus dilutus*. Microcystins can bioaccumulate in numerous taxa, with feeding being the most important route of exposure, but it is suggested that biomagnification is unlikely because of depuration and excretion. Additional studies are needed to determine the ecological relevance of microcystin concentrations in California sediments.

### MP003 Tissue Distribution of Domoic Acid in Field-collected Crabs from the California and Oregon Coasts

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Federal action levels for domoic acid (DA) in seafood drive federal and state efforts to protect human health. Action limits are tissue-specific, with 30 ppm for Dungeness crab viscera and 20 ppm for all other seafood, including Dungeness crab meat. California applies the Dungeness crab viscera action limit of 30 ppm to Rock crab viscera. Existing data, including published studies, show that DA preferentially accumulates in viscera of shellfish and finfish. In limited paired testing in California, DA occasionally has been found in crab meat above the 20 ppm threshold; however, this has only occurred when viscera exceeded the 30 ppm threshold. However, the specific range of DA concentrations in viscera at which the crab meat may exceed the action level has not been determined. In this study, DA in paired meat and viscera of Dungeness and Rock crabs from California and Dungeness crab from Oregon were evaluated to understand the relationship of DA in viscera and meat, and determine whether DA in viscera could be used to predict DA in meat in different species. Our Dungeness crab data show there is a statistically significant positive association between DA in meat and viscera, consistent with the earlier laboratory study for that species (Schultz et al., 2013). Rock crab data show a similar pattern, but the association is not significant. Further evaluation of this relationship could provide a clearer indication of when both meat and viscera need to be tested for DA.

### MP004 Bioaccumulation and toxicity of anatoxin-a: Global review and in vivo bioconcentration study

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Harmful algal blooms (HABs) are worldwide phenomena exacerbated by climate change and nutrient enrichment that can lead to environmental, economic, and human health risks. Anatoxin-a is a common neurotoxin produced by multiple cyanobacterial genera during certain HAB events. It acts as a potent post-synaptic nicotinic agonist, which may lead to convulsions, respiratory paralysis, and death. Anatoxin-a has been shown to bioaccumulate in multiple laboratory and field studies with fish, canines, aquatic plants, avians, and invertebrates. However, little has been done to describe the influence of pH on the bioaccumulation of this ionizable weak base (pKa 9.4). For this reason, uptake and depuration of purified anatoxin-a was evaluated in a model organism, channel catfish (Ictalurus punctatus), exposed at 1 µg/L for 96 h followed by a 96h depuration at two pH levels: 6.5 and 8.5. Fish, water, and plasma samples were taken at 1, 3, 6, 12, 24, 48, 72, and 96h during the uptake phase and at 6, 12, 24, 48, 72, and 96h during the depuration phase. Anatoxin-a was measured using LC-MS/MS. Contrary to previous studies, we observed no anatoxin-a in the fish plasma or tissue during either exposure.

However, the exposure concentration in the current study was two orders of magnitude lower than previous studies. Paired with this analysis, bioaccumulation and toxicity data on anatoxin-a were collated from published studies to identify data gaps and elucidate the potential for anatoxin-a intoxication in various organisms. Data show species specific differences in relation to uptake into various fish tissues with a max of 37.4 µg/g found in Abramis brama gills. In most aquatic plants anatoxin-a was readily taken up, then depurated. Studies with wild avian species found anatoxin-a in liver, gut contents, intestines, and fecal pellets. Anatoxin-a was observed to elicit responses as low as an 0.5 µg/L in the reproduction rate of the rotifer Asplancha girodi. Few studies have looked at chronic effects of anatoxin-a, additive or synergistic effects with other toxins or toxicants, or bioaccumulation in invertebrate organisms. The current study demonstrates that at an exposure concentration of 1 µg/L we do not reasonably expect significant bioaccumulation of anatoxin-a to occur at environmentally relevent pH ranges of 6.5-8.5, and shows multiple data gaps in bioaccumulative and toxicological studies with anatoxin-a.

### MP006 Quantifying cyanoToxin effects on Fathead Minnows using an online monitoring system

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An online monitoring system, AQUATEC, has been developed which assesses behavioral changes in fish with the purpose of detecting toxic events. AQATEC uses video analytics of exposed fish to determine changes in behavior which may indicate the occurrence of stressful conditions. Fish are exposed in a flow through chamber while video of their activity is recorded. Onboard analytics provide real-time analysis of organism and group behaviors. Several parameters are evaluated including orientation, activity, and group dynamics. Experimental work to illustrate the efficacy of such a system by exposing fathead minnows (Pimephales promelas) to copper, microcystins, and prymnesins. An overview of the system, experimental protocol, and initial results will be presented.

### MP007 Acute oral toxicity of Microcystin-LR in mallard ducks

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Harmful algal blooms (HABs ) are becoming an increasing concern worldwide. Microcystin, particularly the microcystin-LR (MC-LR) congener is the most potent and commonly detected cyanotoxin in California. MC-LR is hepatotoxic, nephrotoxic, and carcinogenic, and can be produced by several cyanobacterial species. HABs can affect the health of humans, domestic livestock, pets, and wild animals through direct exposure or bioaccumulation. Among affected animals, aquatic birds are ideal sentinel species for bloom detection and management because they are often the first wildlife to come in contact with toxic cyanobacterial blooms other than aquatic animals. However, clinical and pathological manifestations of cyanotoxicity in birds are poorly understood, and no formal case definition exists. The aims of this study are to (1) quantify the concentration of MC-LR in selected tissue using GC-MS, and (2) localize the tissue distribution of MC-LR via immunohistochemistry in mallard ducks orally exposed to MC-LR. Purified MC-LR (Cayman Inc) was reconstituted with water into four concentrations of MC-LR (0, 1.75, 5.5, and 17.5 mg/Kg). Mallard Ducks were exposed to a single dose MC-LR via oral gavage and subjected to a 14-day observation based on OECD Test Guideline 425, an internationally accepted specification for chemicals testing. Two ducks served as non-exposed control. At 14 days post exposure, blood was collected for a complete blood count and serum chemistry, and animals were humanely euthanized for postmortem examination, histopathology, immunohistochemistry (IHC), and chemical analysis via gas chromatography (GC-MS). In this study, the decreased ambulation observed in low-dose duck but not as prominent in higher dose is similar to a non-monotonic dose-response (NMDR). However, AST in high-dose duck showed 800-fold change but not in lower dose ducks. For immunohistochemistry, Microcystin-LR was localized in several organs (liver and kidney) but was enhanced in the apical region of kidney tubules after 14 days in all ducks exposed to MC-LR, and not found in control. Previous reported oral LD50 of MC-LR in mice is 5 mg/kg. In this study, there is no mortality in duck treated with 17.5mg/kg MC-LR. This suggests that ducks might be more resistant to MC-LR intoxication, but this needs to be further verified.

## Monitoring, Remediation and Ecotoxicological Assessment of Emerging Contaminants in Soil and Water

### MP008 Efficiency of Pharmaceutical and Personal Care Product Removal from Wastewater Effluent by a Constructed Wetland

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Pharmaceuticals and personal care products (PPCPs) are part of a larger group of contaminants of emerging concern that may have an impact on aquatic life. Constructed wetlands, a tertiary wastewater treatment process, have shown potential to be a cost effective, low energy method for removing PPCPs. The Sewanee Wetland is a pilot constructed wetland located in Sewanee, TN, operated jointly by the Sewanee Utilities District (SUD) and the University of the South. Wastewaters at SUD are first screened and aged in two lagoons. The resulting partially-treated wastewater flows into the wetland where photolysis zones, bulrush and pickerelweed plants, sediment adsorption, and microbial activities remove PPCPs from the water. This research project is on-going and will be completed in September 2018. Project goals include measuring the overall removal of PPCPs by the Sewanee Wetlands, determining seasonal or basin-specific differences in efficiency of PPCP removal, and identifying wetland characteristics that correlate with increased removal of PPCPs. Preliminary results show the wetland has low to moderate removal of the 14 PPCPs studied (-22% to 18%). Caffeine (97.74% ± 0.56%) and diphenhydramine (78.60% ± 4.99%) were removed most efficiently. Overall, PPCP removal was greatest during warmer seasons with colder temperatures having a negative effect on PPCP removal. Transformation processes dependent on microbial degradation are predicted to be most affected by reduced temperatures. Plant senescence during winter months may also hamper removal from wastewaters and potentially release compounds to the water column. Further sampling will potentially enable more robust interpretation of seasonal effects, as well as identifying potential factors affecting removal such as plant senescence, sediment disturbance, dilution by rainfall, temperature, retention time, and solar radiation.

### MP009 The occurrence and prevalence of UV filter chemicals in surface water samples in Biscayne Bay and Biscayne National Park

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Recently, much research has been done on the toxicological effects of UV filter compounds found in sunscreen on coral. They have been shown to cause developmental defects in coral planulae and reduce the overall health of reef systems. This research has led to increased public concern and even legislative action to mitigate these adverse effects. However, comparatively little is known about the in situ fate and transport of UV filters. Therefore, this study attempts to determine the actual and theoretical dissipation of UV filters into a typical the water column from a coastal lagoon. Biscayne Bay is an ideal place to conduct this research because it has large and small coral reef systems near a major metropolitan area and many locations along the bay are popular recreation spots where large quantities of UV filters can enter the ecosystem by washing off of swimmers who have applied sunscreen. The prevalence of UV filters in the environment was determined by analyzing water samples from Biscayne Bay using an online solid phase extraction coupled to a triple quad mass spectrometer with an atmospheric pressure chemical ionization source. To determine the range of concentrations that occur in the bay, water samples were taken from locations with varying levels of anthropogenic impact, including in the popular tourist destinations within Biscayne National Park. Eleven compounds were tested for: avobenzone, benzocaine, benzophenone, benzophenone-2, benzoresorcinol, p-benzoylphenol, 4,4'-dihydroxybenzophenone, dioxybenzone, enzacamene, octocrylene, and oxybenzone. The concentrations of these compounds in a preliminary survey of the northern bay ranged from below the detection limits to 23 ppt. Studies were also conducted in a laboratory setting to determine the empirical concentrations of the UV filters by coating an object with different levels of sunscreen formulations and submerging it in a Teflon-lined tank containing artificial and natural seawater.

## Epigenetics and Environmental Exposures: Mechanisms and Effects from Invertebrates to Fishes

### MP010 Assessing the developmental toxicity of the herbicide Ziram in zebrafish (*Danio rerio*)

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Epidemiological studies suggest that environmental exposures to the herbicide ziram, a complex of both zinc and dimethyldithiocarbamate, are associated with an increased risk to Parkinson's disease (PD). The herbicide is also reported to be highly toxic to aquatic organisms, however the mechanisms associated with ziram toxicity and the risk to PD are not fully elucidated. In this study, zebrafish embryos at 6 hours post-fertilization (hpf) were exposed to solvent control (0.1% DMSO, v/v), 1, 10, 100, and 1000 nM ziram for 96 hours. To better define the scope of toxicity, we assessed the effects of ziram on embryonic development, deformities, and larval behavior (light-dark preference). As neurodegeneration and PD are associated with mitochondrial dysfunction and loss of dopaminergic neurons, mitochondrial bioenergetics and the expression of genes in the dopamine system, as well as genes related to oxidative stress, were measured. The results showed that ziram induced lethality in a dose-dependent manner, decreased hatching rate and heartbeat, and caused notochord deformities at 72 and 96 hpf at 100 and 1000 nM. Basal respiration rate of zebrafish at 24 hpf was significantly decreased at 1000 nM ziram, suggesting that ziram negatively impacted mitochondrial bioenergetics in zebrafish embryos. Light-dark preference assays showed that total activity, the velocity in light zone, and total distance moved were significantly increased at 10 nM ziram at 7 dpf. Moreover, ziram decreased the mean time in the dark zone at 1 and 10 nM, suggesting that ziram induced a behavioral response in zebrafish larvae. No significant transcriptional responses of genes related to the dopamine synthesis (tyrosine hydroxylase 1, *th1*),dopamine transporter *(dat)*, dopamine receptors (*drd1, drd2a, drd3,* and *drd4b*), and superoxide dismutase (*sod1* and *sod2*) were detected. Taken together, these data suggest that ziram affects the embryonic development, induce mitochondrial dysfunction, and affect the behavior of zebrafish larvae.

### MP011 Development of a zebrafish hepatocyte assay to study contaminant-induced changes in DNA methylation in fish

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A wide variety of environmental chemicals, including metals, organic contaminants, and pesticides, have been shown to alter levels of DNA methylation in animal cells. However, relatively few studies have address mechanistic linkages between contaminant exposure, DNA methylation, and patterns of gene expression in non-mammalian models. Here, we use a zebrafish liver (ZFL) cell line to investigate contaminant-induced changes in DNA methylation and gene transcription. Initially, ZFL cells were treated with graded concentrations of known methylating (folic acid; FA) or demethylating (5-azacytidine; AZA) agents dissolved in dimethyl sulfoxide (DMSO), and incubated for 24 or 48 hrs. Cell viability was assessed via the resazurin assay in order to determine a range of concentrations of each test chemical that were not overtly toxic to the cells. The highest concentration that was non-cytotoxic was 10 uM for FA, and 0.3 uM for AZA. Global methylation of DNA isolated from harvested cells (n=5 dishes/dose group) was determined via the LUminometric Methylation Assay (LUMA). Significant hypo-methylation of DNA was detected in ZFL cells exposed to the demethylating agent, AZA ($p< 0.0001$). After 48 hrs of exposure, levels of global DNA methylation were 85.0%, 83.3%, and 77.6% for solvent control, 0.1 uM, and 0.3 uM AZA, respectively. DNA isolated from samples treated with the methylating agent, FA, appeared to be partially degraded based on LUMA results and could not be accurately analyzed. The source of this degradation is currently under investigation. ZFL cells were also exposed to the polycyclic aromatic hydrocarbon (PAH), benzo[a]pyrene, with no effect on global DNA methylation after a 24 hr exposure. In addition to measures of global DNA methylation, a qPCR assays was developed for a suite of genes associated with maintenance of DNA methylation in zebrafish, namely *dnmt1, dnmt3a1, dnmt3a2, dnmt3b1, dnmt3b2, dnmt3b3, dnmt3b4, gnmt1, mat1a, tet1, tet2,* and *tet3*. Once fully validated, this ZFL cell assay will be useful for screening chemicals in terms of their potential to disrupt DNA methylation in a model aquatic organism.

### MP012 Epigenetic and genetic mutations in Caenorhabditis elegans after multigenerational exposure to pristine and sulfidized silver nanoparticles

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Adverse effects on reproduction were observed in our previous study after multigenerational exposure of a model organism a nematode *Caenorhabditis elegans* to silver ions ($AgNO_3$), pristine and aged (sulfidized) silver nanoparticles (pA-NPs and sAg-NPs). The toxicity also persisted in the rescued populations after exposure to pAg-NPs and Ag ions. The underlying mechanism of such effects has not been properly investigated. In this study, we investigated whether epigenetic modifications as well as accumulation of genomic mutations can be responsible for the observed increase in the multigenerational reproductive sensitivity. Changes in the histone methylation levels (H3K4me2 and H3K9me3), which were shown to correlate with reproduction, were selected as epigenetic markers for this study. For histone methylation experiments, the exposures were carried out using sub-lethal concentrations ($EC_{30}$ for reproduction) of $AgNO_3$, Ag-NPs and sAg-NPs in simulated soil pore water for 3 generations after which the nematodes were rescued from the exposure for another 3 generations. The levels of H3K4me2 and H3K4me3 were determined using ELISA approach. Significant increases in H3K4me2 levels were observed in response to pristine pAg-NPs. Interestingly, there seems to be no significant response to $AgNO_3$. However, the changes in methylation patterns for H3K4me2 (decrease after exposure and increase after rescue) was different from controls in sAg-NPs. For mutation evaluation, the exposures were carried out for 10 generations with the parent generation ($F_0$) unexposed for all groups. Whole genome sequencing analysis revealed significant increases in the total number of mutations (SNPs, deletions or insertions) in all Ag treatments. Shared only among treatments mutations are indicative of the possible targeted effects of the exposure. This suggests that induced germline mutations may play a role in the increased sensitivity observed previously after multigenerational exposures as this may reduce the fitness of the nematodes. Overall our study demonstrates that different mechanisms of toxicity might be involved in the multigenerational reproduction effects in response to all Ag treatments. Both increases in H3K4me2 levels and mutations seem to be responsible for response to pristine Ag-NPs while only genetic mutations are associated with response to Ag ions. For sAg-NPs, there are changes in the histone (H3K4) di-methylation as well as increases in germline mutations.

**MP013 Genome-wide Assessment of CpG-methylation Changes in Male Fathead Minnows after Exposure to 17α-ethynylestradiol (EE2)**

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17α-ethynylestradiol (EE2) is one of widely-used estrogenic chemicals present almost ubiquitous in aquatic environments throughout the United States and some other countries. EE2, acting as an endocrine disrupting compound, can disrupt hormonal homeostasis, leading to developmental disorders, cancer and other diseases. Exposure to EE2 is known to induce expression of vitellogenin, a precursor protein of egg yolk normally only expressed in female fish, in male fish. However, the underlying epigenomic changes associated with vitellogenin induction and phenotypic changes (e.g. feminization), are not well understood. This study was designed to gain insights into such underlying epigenetic regulation mechanisms by assessing genome-wide DNA methylation changes in CpG sites using the reduced representation bisulfite sequencing (RRBS) technology. Taking advantage of the newly improved assembled fathead minnow genome and annotation in house, we used fathead minnow as the model organism to study DNA methylation changes before and after exposure to EE2. In this study, two groups of male fathead minnow fish, each with 16, were exposed 2.5ng/L and 10ng/L EE2 for 48 hours, respectively. Two additional groups not exposed to EE2 were used control groups: one male group for negative control and the other female group for positive control. We assessed and compared DNA CpG methylation changes immediately after EE2 exposure in both male liver and brain tissues. To address question about whether these methylation changes are temporary or potentially long-term, we also identified and compared methylation CpG patterns of two groups of male fish after 7-day and 14-day depuration of EE2, respectively. This was done separately for liver and brain tissues. Overall, we found that a limited number of CpG regions were subjected to significant methylation changes immediately after EE2 exposures in male liver tissue, and number of affected CpG sites and magnitude of changes were even smaller in male brain tissue. In addition, our results suggest that CpG-sites methylation level could change quickly after exposure to EE2 while most methylation changes may be reversible after depuration of EE2.

**MP014 Impacts Sunlight Imposes on Emerging Rice Field Herbicides and Their Chemical Behaviors in the Presence of Sediment**

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South Louisiana is a unique region where rice fields harvest not only rice but also crayfish, and climate change has increased saltwater intrusion resulting in once freshwater systems becoming brackish water systems throughout the year. Flooded rice paddies serve as shallow water ecosystems for crayfish species, such as *Procambarus clarkii*, to thrive. This puts these macroinvertebrates at potential risk for herbicide exposure, via both the floodwater and the sediment. Newly registered, formulated, or reformulated rice herbicides including benzobicyclon (BUTTE) and quizalofop (Provisia) may be registered for use in Louisiana in the near future and understanding their behaviors is pertinent. Benzobicyclon has previously been shown to rapidly undergo hydrolysis and also photolysis and is potentially persistent in sediments. The photodegradation of each compound will be analyzed in both freshwater and saltwater with and without the presence of rice field sediment to determine laboratory simulations of the shallow rice paddy ecosystems.

**MP015 Medaka as a model for studying transgenerational effects of environmental chemicals and associated molecular changes**

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Health effects of past exposure to environmental chemicals on current and future generations is currently a topic of interest, as the chemicals that were once in the environment do not exist anymore, but it is believed that their effects are being carried by current generations in the form of molecular changes and may be inherited from prior generations. Hence, it is important to develop the tools that can identify the nature of past exposure and at the same time can reliably predict future consequences, particularly in terms of human and ecosystem health. Given that there is an association between epigenetic modifications and past exposure to environmental chemicals, it is possible that epigenetic biomarkers may serve as useful tools to identify the impact of past and current exposures on health issues. Our laboratory is developing a "medaka fish model" to identify common epigenetic biomarkers of the past exposure to environmental chemical contaminants. We have found that embryonic bisphenol A (BPA) and 17alpha-ethinylestradiol (EE2) exposure do not cause immediate phenotypic abnormalities in adulthood but can induce transgenerational reproductive impairment in the third and fourth generations. We also have found that these chemicals induce differential DNA methylation profile (epimutations) in germ cells and the epimutations are transferred to somatic cells in the third (F2) generation. In this talk, we will present a conceptual framework for transgenerational studies and our recent findings of transgenerational health effects and epigenetic modifications associated with reproductive impairment in medaka caused by past embryonic exposure to common endocrine disruptors.

**MP016 Oil-induced methylation of the aryl hydrocarbon receptor repressor (ahrr) promoter in Cyprinodon variegatus**

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DNA methylation, a process by which methyl groups are added to cytosine residues on a DNA molecule, is one of the most studied epigenetic modifications. When gene promoter regions are hypermethylated, the methyl groups act to repress gene transcription; conversely, hypomethylation of promoters can result in enhanced gene transcription. Exposure to environmental xenobiotics is known to induce changes in DNA methylation patterns. One example of this is methylation of the aryl hydrocarbon receptor repressor (*ahrr*) promoter. The *ahrr* is a repressor that acts to inhibit transcription induced by the aryl hydrocarbon receptor (*ahr*) and confer protection against toxic intermediates generated by *ahr* transcription products (such as *cyp1a*). In mammals, exposure to polycyclic aromatic hydrocarbons (PAHs) can result in hypomethylation of the *ahrr* promoter and upregulation of *ahrr* mRNA transcription, providing an important adaptive mechanism to mitigate PAH toxicity. Although the impacts of PAHs on *ahrr* methylation and mRNA expression are understood in mammals, there is little information regarding these effects in fish models. To better understand this system in fish, we exposed larval sheepshead minnows (*Cyprinodon variegatus*, 4 days post-hatch) to 15 ppt sea water or high energy water accommodated fraction (HEWAF) from Deepwater Horizon source oil. Fish were exposed for 48 hours, followed by 48 hours of depuration. Using qPCR, we measured changes in expression of *ahr* related genes (*cyp1a, ahr, ahrr*) to evaluate the magnitude of response to oil exposure. DNA was also extracted and bisulfite treated, and the *ahrr* promoter sequenced to examine methylation patterns. A subset of larvae was reared to adulthood, and DNA from hepatic tissue sequenced to examine whether *ahrr* methylation patterns laid down during development were sustained into adulthood. Adults were then re-exposed to HEWAF to determine whether previous exposure to oil had any impact on the magnitude of response to PAH exposure and whether these responses correlated with *ahrr* promoter methylation patterns. These data provide an important opportunity to understand how promoter methylation regulates the aryl hydrocarbon receptor pathway and elucidate mechanisms governing adaptation to PAH contaminants in fish.

**MP017 Transgenerational epigenetic inheritance and neurobehavioral effects of chlorpyrifos-oxon from developmental exposure in zebrafish (Danio rerio)**

*C. Schmitt, J. Crago, Texas Tech University / Environmental Toxicology / The Institute of Environmental and Human Health (TIEHH)*

Chlorpyrifos is a broad-spectrum organophosphate pesticide widely used in both agricultural and residential applications. Subsequently, chlorpyrifos is a common surface water contaminant in the United States. Larval zebrafish are known to exhibit neurobehavioral alterations after developmental exposure; however, the persistence of effects of chlorpyrifos-exposed zebrafish via generational inheritance has yet to be investigated. In this study, embryos are exposed to chlorpyrifos-oxon at 10 ng/L and 10 µg/L starting at 4 h post-fertilization (hpf) to 5 d post-fertilization (dfp). Behavior assays assessing swimming behavior (distance traveled and velocity) of the exposed germline (F1) are conducted at 5 dpf and 21 dpf, with additional prey-capture assays performed at 21dpf. Data is analyzed using one-way ANOVA with Tukey HSD post hoc test. At 5dpf, samples of replicate treatments are collected in triplicate for later analysis. Remaining F1 embryos are raised to adulthood using standardized laboratory procedures. Each individual replicate and treatment of F1 embryos are spawned separately to produce the epigenetic generation (F2). F2 embryos are not directly exposed to chlorpyrifos-oxon. The subsequent study is repeated to 21dpf for F2 generation, at which time a comparative analysis of F1 and F2 are conducted. All samples collected from F1 and F2 area analyzed for gene expression at the dopaminergic pathway and regulation of global methylation. Ongoing research includes separate analysis of the embryonic activity (twitching) of exposed zebrafish, in situ hybridization, and global DNA methylation.

**MP018 Transgenerational Impact of Synthetic Estrogen on Reproduction in Oryzias melastigma**

*J. Lo, Western Washington University / Department of Environmental Sciences; M. Wan, D.R. Peterson, D.W. Au, City University of Hong Kong / Department of Chemistry*

Estrogenic Endocrine Disrupting Compounds (EEDCs) have the potential to impact an organism by mimicking natural estrogen or binding to its receptors. 17α-ethinylestradiol (EE2) is of particular interest as a synthetic estrogen because of its ubiquity and persistence in aquatic systems. EE2 is known to impair an organism's reproductive fitness and impact fertility rate, hatching success and embryo viability. Studies on the reproductive effect of EEDCs and EE2 have largely focused on the F0 and F1 generations during direct exposure. However, there is a need to investigate the transgenerational impact of these compounds on the F3 generation and beyond as modifications to the germline epigenome can cause these effects on unexposed offspring indicating a longer term impact of the initial exposure. The marine medaka (*Oryzias melastigma*) was used as a fish model to study the effect of EE2 on reproductive fitness using gonadal histology on the F4 generation. Developmental abnormalities were detected in oocytes alongside a decrease in mature spermatocytes across the high concentration exposures for the long term parental exposure group. Although this trend was established, additional work is needed to address additional transgenerational effects, particularly the underlying molecular, biochemical and physiological changes that occur to impede an organism's ability to reproduce.

**MP019 Transgenerational toxicity of biocide, CMIT/MIT in Daphnia magna: Epigenetics and proteomics analysis**

*J. Gim, N. Chatterjee, University of Seoul; J. Choi, University of Seoul / School of Environmental Engineering*

Epigenetics is the study of mitotically heritable or inheritable changes induced without alteration of DNA sequence. Such changes in diverse species, including cladocerans, have been reported to be induced by environmental stress factors and could be transgenerationally inherited without direct exposure at the descendant generation(s). Besides, proteomics is among one of the OMICS studies which can provide the snapshot of all the altered mechanisms of its phonotype. CMIT/MIT(chloromethyliso thiazolinone / methylisothiazoline) is a known biocide and widely used in water treatment processes and thus has a potential risk of an aquatic ecosystem. To this end, we have performed the transgenerational as well as multigenerational effects of CMIT/MIT in Daphnia magna in respect of epigenetic and proteomic context. To fulfil the aim of study, we employed multigenerational and/or transgenerational exposure (P0-F10) and multi-level endpoints from molecular to eco-physiological levels. We found CMIT/MIT caused changes in reproduction, behaviour, body size-spine length, heart rate, lipid contents, DNA damage, DNA methylation and in proteomics level. Taken together, our results showed that CMIT/MIT has transgenerational impacts on Daphnia magma which was observed through integrated molecular-physiological endpoints. Acknowledgement : This work was supported by the Mid-career Researcher Program (2017R1A2B3002243) through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning.

**MP020 Functional implications of altered miRNA following a multiple stressor exposure in zebrafish (*Danio rerio*)**

*H. Ikert, B. Katzenback, P. Craig, University of Waterloo / Biology*

As the human population continues to grow, increased anthropogenic stress is placed on the aquatic environment and the need to understand the effects this will have on aquatic organisms is rising. Pharmaceuticals such as venlafaxine (VFX), a heavily prescribed and readily detectable antidepressant, are found downstream of wastewater treatment plants. As a result of climate change, increased surface water temperatures and decreased dissolved oxygen levels have been observed. In this study, a multi-stressor approach was used to determine the cumulative, sublethal effects of the aforementioned stressors on microRNA (miRNA) and proteins in adult zebrafish (*Danio rerio*). MiRNA are small, conserved, non-coding RNA which act by decreasing mRNA translation. This impacts the functional responses of downstream targets and is a method of environmental and epigenetic regulation of phenotypic responses. In order to determine functional consequences of changes in miRNA abundance, protein abundances can be quantified. Adult zebrafish were exposed to control (27°C, 100% $O_2$, 0 µg/L VFX) or stressed (32°C, 50% $O_2$, 1.0 µg/L VFX) conditions for 24 hours (acute) or 21 days (chronic). RNA was extracted from liver, gonad, and muscle tissue and RT-qPCR was performed on specific miRNA related to proteins that respond to hypoxia, heat stress, or contaminants. The same tissues were used for protein quantification using iTRAQ (isobaric tags for relative and absolute quantification). This comparison between stressed and non-stressed fish demonstrated altered miRNA and protein abundance. Interestingly, miRNA changes were different between sex, tissue, and length of exposure. This functional linkage of miRNA response to downstream, target protein abundance is crucial in understanding how epigenetic responses to environmental disruption affect fish health and fitness.

## Exposure Assessment and Modeling for Ecological Risk Assessment of Veterinary Pharmaceuticals and Pesticides

**MP021 Global scanning of selective serotonin reuptake inhibitors in the environment: Occurrence, hazards, and the impact of wastewater treatment**

*R. Mole, University of North Carolina Wilmington / Mathematics; B.W. Brooks, Baylor University / Department of Environmental Science*

As the world's population becomes more concentrated in and around urban centers, resource consumption and pharmaceutical use are also increasing, with wastewater infrastructure unable to meet growing demands. Urbanization is increasingly impacting aquatic ecosystems and potential hazards of pharmaceuticals introduced by wastewater effluents must be considered. In the present study, a global hazard assessment of selective serotonin reuptake inhibitors (SSRIs) was performed across all geographic regions and multiple matrices. A comprehensive literature search was completed and SSRI occurrence data was collected and collated resulting in 152 individual publications. Across all matrices, the majority of publications came from Europe (50%) followed by North America (38%) and Asia-Pacific (10%). There were minimal to no publications from many developing regions of the world such as Latin America, Africa, and South America. Environmental exposure distributions (EEDs) $5^{th}$ and $95^{th}$ percentiles for all SSRIs across all geographic regions were 2.31 and 3022.08 ng/L for influent, 5.25 and 841.60 ng/L for effluent, 0.81 and 127.70 ng/L for fresh water, and 0.51 and 22.31 ng/L for salt water, respectively. To estimate the potential hazards of SSRIs in the aquatic environment, individual therapeutic hazard values (THVs) of SSRIs were calculated, and the predicted percent exceedance was calculated across all geographic regions. In influent and effluent, sertraline is expected to exceed the THV 49% and 29% of the time, respectively, demonstrating the need to better understand the potential hazards of SSRIs in aquatic ecosystems. Similar assessments were also completed for effluent detections among various types of wastewater treatment technologies to investigate whether different technologies differentially impact SSRI occurrence. This unique global approach highlights the usefulness of global assessments to identify compounds requiring more toxicological attention, as well as regions where more monitoring and management are necessary.

**MP022 Identifying estrogenic and anti-androgenic endocrine disrupting compounds: Can sexual maturity status influence test outcome?**

*K. Roush, M.K. Sellin Jeffries, Texas Christian University / Department of Biology*

Exposures to some veterinary pharmaceuticals and pesticides pose a threat to the health of humans and wildlife, as some have been identified as reproductive endocrine disrupting compounds (REDCs) capable of altering sexual development, reproductive success and behavior. As such, the goal of this project was to determine whether sexual maturity status, as assessed via the prominence of secondary sexual characteristics, in male fathead minnows (*Pimephales promelas*) influences their response to REDCs. Specifically, we sought to determine whether less sexually mature males differed from more sexually mature males in their responses to 17β-estradiol (E2, a common human and veterinary pharmaceutical) and vinclozolin (VZ, a fungicide). Adult male fathead minnows were sorted by the prominence of secondary sexual characteristics, exposed to E2 or VZ for 7 days and sampled for the analysis of estrogen- and androgen-responsive gene expression. Results showed that fish with highly prominent secondary sexual characteristics responded differently to REDCs than fish with less prominent secondary sexual characteristics indicating that sexual maturity status influences REDC exposure outcome. For example, an increase in hepatic vitellogenin expression was detected in the more sexually mature males but was not detected in the less sexually mature males after exposure to E2; therefore, had less sexually mature males alone been utilized, the estrogenic effects of E2 would not have been identified. These results indicate that sexual maturity status should be taken into consideration in the implementation of screening assays as failure to utilize fish of the "appropriate" maturity status can skew test results. Ultimately, by refining existing screening assays to ensure adequate detection of pharmaceutical and pesticide REDCs, environmental assessments of these compounds will improve.

**MP023 Ocurrence and spatial-temporal trends of monensin in a mixed-uses watershed in Argentina**

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Occurrence and potential ecological risk of veterinary medicines in aquatic ecosystems of South America, is not well reported. This work attempts to generate relevant data about the occurrence, levels and spatial-temporal trends of monensin (MON) in a mixed-uses watershed of Argentina. El Pantanoso is a first-order stream located in the southeast of the Buenos Aires Province, with a catchment area of 476 $km^2$. This watershed is influenced by urban and farming activities. There is a Constructed Channel (CC) that receives the wastewater plant treatment (WWPT) discharge of Balcarce City and effluents of a feedlot production; and it is coupled to the stream in the main channel. Water samples were collected from 11 sampling sites in August and November 2017 covering the high flow and low flow periods, respectively. Sampling sites were chosen to cover from the headwaters to the end of stream in the floodplain contemplating different possible points of pollution by veterinary medicines. Samples was collected by triplicate using 1 L polypropylene bottles and stored at -20°C until analysis. Samples were analyzed using liquid chromatography coupled to a tandem mass spectrometer. The limit of detection (LOD) was set in 0.01 µg/L and the limit of quantification was estimated similarly to the LOD. MON was detected in 90% and 100% of the samples in August and November, respectively. In August, at headwaters and floodplain MON was detected at minimum levels (0.2 ± 0.03 µg/L) associated with the extensively livestock production. Highest MON levels were detected associated with cattle slaughter waste water effluent (2.6 ± 0.74 µg/L). In the CC, the levels varied from non detected at the beginning with urban and periurban influence, to 1.0 ± 0.1 µg/L downstream to the WWPT, and 0.6 ± 0.03 µg/L downstream to the feedlot production. In November, the levels no surpass 0.02 µg/L at different sites. MON occurrence become an ubiquitous emergent contaminant in this watershed. Cattle slaughter was the main point source pollution of MON and the WWPT result the secondary source of impact. The highest concentrations found close to the cattle slaughter in August could be explained for the intense rains during this period that induced a strong drainage from the treatment lagoon to the stream, because the distance between both sites is less than 0.5 km. This is the first report that describes the presence of monensin in surface waters of Argentina.

## Bayesian Network Applications for Environmental Risk Assessment and Management

**MP024 Assessing Risk at the Landscape Scale: Metapopulation Models as the Endpoint of a Bayesian Network-Relative Risk Model**

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Populations are often the endpoints of interest in Ecological Risk Assessments (ERAs). However, many population-level ERAs do not integrate metapopulation dynamics, such as dispersal between subpopulations, and thus preclude an understanding of how population viability is affected by toxicants and environmental stressors at the landscape scale. In this study, Chinook salmon (*Oncorhynchus tshawytscha*) metapopulation models were integrated as the endpoint of a Bayesian Network- Relative Risk Model (BN-RRM) to assess the risk of organophosphate exposure and ecological stressors to multiple subpopulations across multiple seasons in an agriculturally impacted watershed. A stochastic matrix metapopulation model was developed using demographic data for three locally adapted spring Chinook salmon subpopulations and one hatchery subpopulation in the Yakima River Basin (YRB), WA. This model was simulated for 24 exposure scenarios, each with 200 simulations of a 50-year metapopulation projection. Subpopulation size outcomes were incorporated as the endpoint of the BN-RRM, which integrates the effects of toxicological (organophosphates) and ecological (water temperature and dissolved oxygen) pathways on Chinook salmon into a single probabilistic ERA. Risk was defined as the probability that a subpopulation would decline from the initial abundance (500,000 individuals). Results of this ERA indicated that risk for wild spring Chinook subpopulations was higher (97% for the American River and 74% for the Naches River subpopulation) than for non-wild subpopulations experiencing high dispersal with a local hatchery (30% for the hatchery and hatchery-supplemented subpopulations). A seasonal effect of stressors, driven primarily by high water temperatures in summer, was also detected where summer had the highest risk (68.7%) and winter had the lowest risk (52%). Ecological stressors made up a greater proportion of total risk in most scenarios than exposure to organophosphates. These results suggest that metapopulation spatial structure, local adaptation of subpopulations, and seasonal differences in exposure to stressors impact the distribution of risk at the landscape scale. This method of using site-specific metapopulation model simulations as a probabilistic ERA endpoint shows promise for estimating the spatiotemporal impacts of stressors on Endangered species at the metapopulation level, where metapopulation dynamics and spatial structure create complex risk dynamics.

**MP025 Bayesian network model for risk assessment based on fish embryo testing: A probabilistic weight-of-evidence approach**

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Reduction of animal testing wherever possible is requested by EU Directive 2010/63/EU. Fish Embryo Toxicity (FET) testing can be an alternative to using juvenile fish in acute toxicity testing. However, FET data are currently not accepted as a replacement to juvenile fish acute toxicity data for regulatory purposes such as REACH, without sufficient weight of evidence (WoE). The development of a WoE approach for FET data has been recommended by the European Chemicals Agency to significantly reduce the number of animals required for hazard assessments of chemicals. We propose a Bayesian network (BN) modelling approach for quantifying the lines of evidence demonstrating the relationship between FET data and juvenile fish toxicity results. BN is a probabilistic modelling methodology which is an increasingly used in ecological risk assessment as well as in environmental research and assessment more generally. The purpose of the proposed BN model is to integrate information from large ecotoxicological and physico-chemical datasets, and apply it in a WoE approach to predict fish acute toxicity of chemicals from data on fish embryo toxicity testing in combination with other predictive information. The current model has been developed from data on fish embryo and juvenile acute toxicity for more than 200 chemicals, including QSAR (n=197), LC50 for embryo (n=541) and juvenile fish (n=1459), and EC50 for algae (264) and Daphnia (n=2328). The toxicity values (EC50 and LC50 values, in mg/l) are discretized to 5 levels: very low (>100), low (5-100), medium (0.5-5), high (0.01-0.05) and very high (< 0.01). The links between nodes are quantified by conditional probability tables (CPT), where the values are obtained by two methods: counts of observations or expert judgement. The BN predicts the toxicity level of each chemical to juvenile fish by combining information in four pathways: (1) fish embryo toxicity, (2) physical and chemical properties, (3) toxicity to fish of other chemicals in the same category, and (4) toxicity to other species (algae and Daphnia). This WoE approach is being validated by comparing the BN model predictions with observed data for juvenile fish acute toxicity of each chemical. This BN model can be used to assess the risk of various contaminants to fish based on fish embryo toxicity data in combination with other available information without the need to perform fish acute toxicity testing.

**MP026 A Bayesian network approach to refining terrestrial risk assessments: Mercury and the Florida panther (*Puma concolor coryi*)**

*M.G. Barron, USEPA / Gulf Ecology Division; J.F. Carriger, Jr., USEPA / National Risk Management Research Laboratory*

Traditionally hazard quotients (HQs) have been computed for ecological risk assessment, often without quantifying the underlying uncertainties and variability in the risk estimate. We demonstrate a Bayesian network approach to quantitatively assess variability and uncertainties in HQs using a retrospective case study of mercury (Hg) risks to Florida panthers (*Puma concolor coryi*) based on Barron et al. (*2004. Ecotox 13:223*). The Florida panther is the last remaining wild puma population in the eastern U.S. Mercury exposure has been hypothesized as a contributing factor to population decline and individual panther deaths across the South Florida ecosystem. A tiered approach with Bayesian networks was developed to identify and quantify the causal risk factors, propagate uncertainties in causal relationships, and examine the refinements necessary for predicting the overlap of Hg exposure and effects distributions to calculate HQs. Multiple HQs for the Florida panther and Hg risks were calculated with the Bayesian network to examine potential scenarios based on changes to causal exposure factors. Application of Bayesian networks in the computation of HQs provides a transparent and quantitative analysis of uncertainty in risks.

## Advancing the -omics into Regulatory Frameworks: Case Studies and Perspectives

**MP027 Building Bayesian Network model for identification of causal relationships between KEs via TGF-beta signaling pathway**

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Defining causal pathway is a critical step for application of adverse outcome pathway (AOP) on the risk assessment. A Bayesian Network (BN) is a model that presents probabilities of the dependencies between any two variables in a graphical form. It is capable of diagnostic, predictive, and inter-causal reasoning. Transforming Growth Factor-beta (TGF- β) is a central modulator of inflammation and fibrosis. To identify causal relationships between key events (KEs) in an AOP of fibrosis via TGF-β signaling pathway, we built BN structure from expert knowledge. The BN structure consists of oxidative stress, endoplasmic reticulum stress, DNA damage, inflammation and Fibrosis. We used multi-walled carbon nanotubes (MWCNTs) as potential stressor. qRT-PCRtest were conducted on the various biomarkers related to each KE using human bronchial epithelium cell (BEAS-2B) to generate the gene expressions dataset for learning the BN model. The BN model was learned using *bnlearn* package in R statistical computing environment. The preliminary results showed that BN model has potential for identification of cause-effect relationships between the parameters. For further study, we are planning to do various dose- and time response test (using qPCR and ELISA) for missing data, so that we can integrate additional data with the BN model for increasing

accuracy and predictive power of the BN model. Acknowledgement: This work was supported by a grant from the Korean Ministry of Environment through 'Environmental Health R&D Program' (2017001370001)

**MP028 Transcriptomics for Elucidating Effects of Carbon and Boron Nitride Nanomaterials in *Pseudomonas aeruginosa***

*M. Mortimer, N. Devarajan, D. Li, P. Holden, University of California, Santa Barbara*

Development of environmental and agricultural applications of engineered nanomaterials (ENMs) requires thorough knowledge of ENM biological effects, including microbial responses. We found that the specific growth rate (aerobic, 30°C) of wild type *Pseudomonas aeruginosa* PG201, an environmentally and clinically relevant bacterial strain, was not affected by up to, and including, 10 mg/L of multiwall carbon nanotubes (MWCNTs), graphene, boron nitride flakes (BN) or carbon black (CB) amended to mineral medium. Although bacteria-ENM agglomerates were formed, no significant cell membrane damage was detected by propidium iodide/SYTO9 staining. However, transcriptional analysis by next-generation sequencing indicated differential regulation of 111, 44, 26, and 25 genes ($p < 0.05$; $\log_2$(fold change) $\geq 2$) in MWCNT, graphene, BN and CB treatments, respectively, compared to the control culture without ENMs. MWCNTs, which induced the most pronounced transcriptomic response, caused the upregulation of genes encoding general stress response, sulfur metabolism, and transport of small molecules and downregulation of genes encoding flagellar basal-body rod proteins and other virulence-related factors, nitrogen metabolism, and membrane proteins. In addition, *czcR* and *czcS*, the genes of a two-component regulatory system associated with quorum sensing, heavy metal and antibiotic resistance, were downregulated in MWCNT-exposed bacteria. Consistently, antibiotic susceptibility tests of MWCNT-treated *P. aeruginosa* showed that the minimal inhibitory concentrations (MICs) of meropenem and imipenem decreased twofold, indicating increased susceptibility of *P. aeruginosa* to two last-resort antibiotics. In summary, we show that sensitive transcriptional end points shed light on subtle physiological effects of ENMs that may be predictive of adverse outcomes.

**MP029 Building Bayesian Network model for identification of causal relationships between KEs in oxidative stress and inflammation related AOP**

*J. Jeong, S. Bae, University of Seoul / School of Environmental Engineering; C. Lim, Chung-Ang University / Department of Applied Statistics; J. Choi, University of Seoul / School of Environmental Engineering*

Defining causal pathway is a critical step for application of adverse outcome pathway (AOP) on the risk assessment. A Bayesian Network (BN) is a model that presents probabilities of the dependencies between any two variables in a graphical form. It is capable of diagnostic, predictive, and inter-causal reasoning. To identify causal relationships between key events (KEs) in an AOP of fibrosis via oxidative stress and inflammation signaling pathway, we built BN structure from expert knowledge. The BN structure consists of oxidative stress, DNA damage, inflammation and Fibrosis. Various environmental chemicals, such as, biocides and nanomaterials, were used as potential stressors. qRT-PCRtest were conducted on the various biomarkers related to each KE using human bronchial epithelium cell (BEAS-2B) to generate the gene expressions dataset for learning the BN model. The BN model was learned using *bnlearn* package in R statistical computing environment. The preliminary results showed that BN model has potential for identification of cause-effect relationships between the parameters. For increasing accuracy and predictive power of the BN model, extensive dose- and time response analysis needs to be performed. Acknowledgement: This work was supported by a grant from the Korean Ministry of Environment through 'Environmental Health R&D Program' (2017001370001).

**MP030 A network approach to quantify the relationship between individual contaminants and biological responses in environmental mixture studies**

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Interpreting biological effects caused by exposure to chemical mixtures is an ongoing challenge in ecotoxicology. Exposure assessments utilizing transcriptome profiling have been valuable for characterizing the effects of environmental mixtures on aquatic organisms. However, a gap remains between these assessments and the specific information needed to make regulatory and management decisions. New approaches are needed to identify the specific mixture constituents that are contributing most strongly to adverse outcomes. Here, we leverage existing chemical and transcriptomic datasets to estimate the association between the occurrence and concentration of individual chemicals and transcriptomic changes following exposure. Transcriptomic and chemical data come from experiments where male fathead minnows were exposed in situ to water from multiple locations in the Shenandoah River watershed. Exposure water was analyzed for over 550 contaminants and hepatic gene-expression profiles were measured using microarrays. Both data-driven and hypothesis-driven analyses are used to establish linkages between contaminants and biological effects in organisms. Our hypothesis-driven approach uses databases of chemical-gene interactions to develop hypotheses about the patterns of differential gene expression that are expected for an individual chemical. These hypotheses are then tested by comparing expected patterns to observed patterns in exposed organisms using Gene Set Enrichment Analysis. Significant enrichment of a gene set for a given chemical indicates that there is similarity between the expected and observed patterns of gene expression, and suggests a causal role for that chemical. The data-driven approach measures correlations between detected contaminants and individual genes. The resulting correlation matrix was used to build a bipartite network and to identify the contaminants with the highest degree (significant correlation to gene expression). Other network metrics, such as modularity and centrality, are expected to be informative for grouping chemicals with similar mechanisms of action and identifying the potential for additive mixture effects. These two approaches will help identify the subset of environmental chemicals that pose the most risk to aquatic organisms, which is necessary for conserving aquatic habitats.

**MP031 Harnessing Chemical Proteomics to Elucidate Molecular Mechanisms of Toxicity of Water Disinfection By-products**

*D. Hall, University of Toronto / Chemistry; K. Yeung, University of Toronto; H. Peng, University of Saskatchewan / Department of Chemistry*

Disinfection by-products (DBPs) from chlorine disinfected drinking water have been epidemiologically linked to a myriad of health effects including bladder cancer and premature-births. Yet, current regulations fail to account for hundreds of known and unknown DBPs — many of which are suspected to be the true drivers of toxicity. Our previous studies have shown that Nrf2-mediated oxidative stress is the primary toxic pathway of DBPs. Motivated by this, we further investigated the toxic mechanisms of two classes of prominent DBPs: halogenated acetic acids and acetamides. Cytotoxicity and oxidative stress assays revealed iodinated DBPs to be the most toxic, followed by brominated, then chlorinated DBPs. Consistent to these results, iodinated DBPs also exhibit the greatest capacity for protein binding via covalent cysteine modification. We elucidated the exact protein targets using Affinity-Based Protein Profiling chemical proteomics. We found hundreds of proteins to be covalently modified by DBPs with compound specific-patterns. Potential impacts of such modifications on protein conformation changes and functions were further evaluated using our Target Identification by Ligand Stabilization proteomics method. Molecular insights from our work provided the first evidence that covalent modification of cysteines on cellular proteins is the primary pathway by which DBPs exhibit oxidative stress-mediated toxicities. Furthermore, the fundamental research derived in the present study motivated us to develop a chemical reactivity probe for high-throughput assays to identify toxic components of DBPs from real water samples.

**MP032 Predicting adverse outcomes of selenomethionine exposure to embryonic white sturgeon (*Acipenser transmontanus*) using in-ovo microinjection**

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Selenium (Se) is a trace element and nutrient for almost every form of life. However, it has a narrow margin between essentiality and toxicity that can be surpassed when anthropogenic activities cause elevated Se concentrations in aquatic environments. This toxicity occurs when primary producers biotransform the excess Se to selenomethionine (SeMet), a bioaccumulative methionine analogue that can be maternally transferred to the eggs of oviparous species, leading to impairment, or complete failure, of recruitment. The primary mechanism identified for SeMet toxicity is redox cycling of the metabolite methylselenol, yet not all species exposed to SeMet show signs of oxidative stress. While SeMet exposure poses potential risk to many oviparous vertebrates, the white sturgeon (*Acipenser transmontanus*) is at particular risk due to its SeMet sensitivity, life history, and endangered status. Though tissue specific (ex. egg, ovary) concentration guidelines have been employed by the United States Environmental Protection Agency, these thresholds are only capable to indicating imminent or present risk. Protection efforts could therefore be enhanced through the development of more sensitive tools capable of predicting the risks that have yet to manifest. The purpose of this study is to identify the molecular initiating event of and possible key events leading to SeMet toxicity in the white sturgeon. In order to characterize this pathway 1488 white sturgeon embryos were exposed to either negative or process control conditions, or graded SeMet injection (nominal: 8.8, 13.3 and 20 µg/g dry mass) conditions *in ovo* in four replicates to determine the potential effects of SeMet exposure in early life stage white sturgeon. Embryos were sampled five days pre and post-hatch for transcriptomic analyses, and at swim up for biochemical, histological, and apical endpoint analyses in order to delineate the causal chain of toxic events across levels of biological complexity. The results of this experiment will aid in developing a highly sensitive predictive model for SeMet toxicity and will hopefully enhance the assessment of Se risks posed to white sturgeon.

## Environmental Stewardship in a Changing Climate – An Integration of Engineering and Environmental Science

**MP033 Ecosystem services accounting in support of corporate environmental stewardship in a changing climate**

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Corporations face increasing pressure from shareholders and the public to account for environmental impacts from operations. Carbon sequestration credits (i.e., “green” or “blue” credits) are one option for offsetting greenhouse gas emissions by purchasing units of forest, mangrove, or wetland habitats, for example, but these credit projects provide additional valuable ecosystem services (e.g., biodiversity protection, flood control, contaminant sequestration, etc.) that are not incorporated into the carbon sequestration credit model. These ecological services may be of value to corporations considering purchase of such credits, and hence there is a need to understand, quantify, and track these additional ecological benefits. There is also a need for crediting agencies and credit purchasers to understand the differential risks associated with various offset projects (e.g., will climate-driven sea-level rise inundate a wetland bank before credit gain is maximized?). Finally, businesses need to weigh costs/benefits of offsetting options against consideration of other factors. For example, carbon sequestration can be performed anywhere, but there may be greater ecological benefits in some locations than others. Facility risk may be reduced, and resiliency subsequently increased, if the offsetting is done at the source of the emissions. All these factors need to be considered in a holistic assessment of the full suite of ecological benefits/risks of a carbon-offset project over the life-cycle of the designated offset. Here, we discuss how an ecosystem services valuation tool (EcoAIM) can be used to (1) quantify the ecological benefits that accrue from various credit/mitigation projects while incorporating user preferred services and (2) incorporate climate risk model scenarios to assess future climate-related changes on credit/mitigation projects and industrial operations. In this presentation, we use a hypothetical example to show how a corporation considering investing in mitigation offsetting can use a proactive risk management approach to assess future benefits and liabilities from candidate mitigation projects.

**MP034 Global climate change and its influence in planning remediation, restoration and long-term management of contaminated sites**

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Global climate change (GCC) and the other effects of adding carbon dioxide to the atmosphere already is having and will continue to have impacts on the remediation, restoration and management of contaminated sites. The expectations are of more severe storm events, changes in precipitation amount and type, sea level rise, and ocean acidification. Given the long periods of liability for a contaminated site, often multiple decades, these changes in prevailing conditions will affect both the engineering of restoration process and the surrounding ecological structure. Therefore, GCC requires a revision of the current approaches to risk assessment and decision making. Landis et al (2013) proposed seven principles that should be followed when considering remediation actions and the supporting ecological risk assessment activities. Principle 1: Consider the importance of GCC-related factors in the ERA process and subsequent management decisions and make them appropriate to the spatial and temporal scales. Principle 2: Assessment end points should be expressed as ecosystem services. Principle 3: Responses of the ecosystem services (endpoints) can be positive or negative. Principle 4: The ERA process requires a multiple-stressor approach, and responses may be nonlinear. Principle 5: Develop conceptual cause–effect diagrams that consider relevant management decisions as well as appropriate spatial and temporal scales to allow consideration of both direct and indirect effects of climate change. Principle 6: Determine the major drivers of uncertainty, estimating and bounding stochastic uncertainty spatially and temporally, and continue the process as management activities are implemented. Principle 7: Plan for adaptive management to account for changing environmental conditions and consequent changes to ecosystem services. In the five years since the publication of these principles methods now exist to incorporate each into an appropriate risk assessment and decision making process to manage contaminated sites. Methods to address each of these principles are outlined in the presentation with examples from case studies.

**MP035 Incorporating a framework for risk assessment, risk management, and risk mitigation of extreme weather events at Superfund sites**

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More than 300 active Superfund sites are located in areas that are prone to flooding or vulnerable to sea-level rise. Increasing frequency and intensity of extreme weather events may pose severe threats to human health and the protectiveness of remedies at those Superfund sites located in high flood risk areas. Widespread flooding from Hurricane Harvey demonstrated the risks for contaminated sites: thirteen Superfund sites flooded or were otherwise damaged during the storm. At one of those sites, the San Jacinto River Waste Pits, a temporary cap covering pits with dioxin-contaminated sediments was compromised, exposing the waste material in the pits. Potential impacts from extreme weather events can include physical and operational disruptions: power outages; physical or water damage to remedial components such as caps, pumps, or onsite treatment systems; reduced access; increased erosion; or sediment deposition. Sediment erosion and/or deposition will also challenge evaluating performance or recovery as baseline or reference area conditions may change as a result of extreme weather impacts. Currently, USEPA guidance for conducting remedial investigations and feasibility studies (RI/FS), remedy design, and five-year reviews of Superfund sites does not include specific advice on how to consider extreme weather effects and, particularly, the increasing intensity and frequency of such events on the protectiveness of selected remedies. Financial impacts may include increased remedy costs resulting from characterization of impacts, repair of remedial components, or even re-design of remedies or retirement of damaged remedial components. Understanding the effects of extreme weather events necessitates a vulnerability analysis that is incorporated as early as possible in the Superfund site lifecycle. In this presentation, we will discuss potential impacts from extreme weather events on Superfund sites: impacts on operations, on the protectiveness of remedies, and on baseline characterization. Additionally, we will demonstrate a framework that can be used to evaluate extreme weather event impacts at sites at various stages of the Superfund site lifecycle, including data collection and modeling activities that responsible parties can take for sites in the RI/FS phase and mitigation and adaptation measures that existing sites can incorporate.

## Immunotoxicology: Identifying Adverse Effects, Developing New Approaches and Confronting Existing Challenges

**MP037 Effects of UV-234 and UV-320 on mitochondrial bioenergetics and immune response genes in the zebrafish (*Danio rerio*)**

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Among the benzotriazole ultraviolet stabilizers (BUVSs), UV-234 and UV-320 are two of the most frequently detected chemicals in aqueous ecosystem. Despite some evidence for bioaccumulation in aquatic organisms, limited toxicological data on these compounds have been generated in fish. In the present study, zebrafish embryos were exposed to 0.01, 0.1 and 1µM UV-234 or UV-320 for up to 6 days. Developmental toxicity of UV-234 and UV-320 was assessed, as well as their effects on mitochondrial bioenergetics and immune response-related gene expression as there is a close association between the mitochondria and the immune system. In the UV-234 treatment, hatching time of zebrafish embryos was significantly increased compared to controls. Non-mitochondrial respiration and mitochondrial respiration of zebrafish embryos were significantly decreased at 1µM UV-234 group after 24 and 48 h exposure, respectively. There were no changes in the expression of the investigated key immune regulators, chemokines and cytokines (*tlr5a, tlr5b, mmp9, il8, tnfa, cxcl-C1c, nfkb1*, and *ifng*). In the UV-320 treatment, hatching time of zebrafish was not affected but both basal and non-mitochondrial respiration (both at 48 hours) were significantly higher in the 1 µM group compared to the control group. Moreover, the mRNA levels of chemokines (*il8* and *cxcl-C1c*)were suppressed in response to 0.1 µM UV-320 showing immunomodulatory potential of UV-320. Taken together, these data suggest that UV stabilizers may act to modulate mitochondrial bioenergetics and the immune system in developing embryos. Future studies will be aimed at discerning the interaction between mitochondria and the immune system in response to UV stabilizers

**MP038 Endocrine Disrupting Compounds and Immunity: The Effects of Estrogens and Antiestrogens on Immune Function in Fathead Minnows**

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Estrogenic endocrine-disrupting compounds (EDCs) are ubiquitous in the aquatic environment, posing potential threats to wildlife. Research on estrogenic EDCs has focused primarily on effects on the reproductive system. However, sex steroids also affect other body systems, such as the immune system. Though few studies have sought out to determine the effects of estrogenic EDCs on immunity, known differences between male and female immune function suggest a role for sex steroids. While it is agreed that estrogens have the capacity to alter immunity in teleosts, the effects of these alterations on the organism are not currently understood. The current study both analyzed the effects on immune function of male fathead minnows (FHM) exposed to estradiol, as well as the effects on immune function of female FHM exposed to the anti-estrogen fadrozole. The effects in each group were assessed by whole-organism (i.e., pathogen resistance), tissue (i.e., spleen index), cellular, (i.e., leukocyte counts) and molecular endpoints (i.e., expression of genes associated with immune function). First, the results revealed differences between control males and control females with regard to pathogen resistance and the expression of a subset of immune-related genes, confirming previously noted differences in male and female immune function. Regarding the effects of estrogens and anti-estrogens on immunity, gene expression results suggest that the complement pathway may be under the influence of estrogens. However, estrogens and anti-estrogens do not appear to influence immune function at the tissue or whole organism level. Overall, the results of the current study point to the sexually dimorphic nature of the immune system and suggest that exposures to estrogens and/or anti-estrogens may alter some aspects of immunity. In light of these results, future studies aiming to analyze the effects of estrogen exposure on aquatic wildlife should consider potential impacts on the immune system.

**MP039 The impacts of early life stage hypothyroidism on immune function and the immune response in the fathead minnow (*Pimephales promelas*)**

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Current evidence suggests that thyroid hormones (THs) play a role in immune function and the immune response. For example, TH receptors are expressed in immune-related tissues such as spleen and head kidney, and several types of immune cells, such as dendritic cells and lymphocytes, are capable of producing thyrotropin. There is also evidence that THs may impact the development of the immune system as organisms that experience thyroid suppression during immune development have been shown to experience alterations in the immune response and pathogen resistance during adulthood. However, the number of studies that explore the role of THs in immune development is limited, and the mechanisms leading to any alterations in overall immune function are poorly understood. It is important to elucidate the role of THs in immune development given that many environmental contaminants have been shown to disrupt

TH homeostasis and may also have negative impacts on the immune system. As such, the main goal of this study was to determine the long-term consequences of early life stage hypothyroidism on immune function. To achieve this goal, fathead minnows (FHMs, *Pimephales promelas*) were exposed to the model thyroid suppressant propylthiouracil (PTU) from Yersinia ruckeri via intraperitoneal injection and monitored for 14 days to determine pathogen resistance. At eight hours post injection, a subset of minnows was sacrificed for the determination of spleen index (spleen mass relative to total body mass), hematocrit (ratio of volume of erythrocytes to total blood volume) and bacterial load (abundance of a *Y. ruckeri* specific transcript in splenic tissue). Unexpectedly, no statistically significant alterations were detected for any of the endpoints measured, including pathogen resistance, suggesting that early life stage hypothyroidism does not impact immune function or the immune response during adulthood. Yet, it is important to note that only a single host-pathogen model was used in this study, and the consequences of a viral or parasitic infection remain unknown.

## Ecotoxicological Impact of Multiple Stressors in Aquatic Ecosystems

**MP040 Impacts of wastewater discharges on freshwater mussels in a watershed within Southern Ontario, Canada**

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Wastewater treatment lagoons serving communities across rural Canada were originally constructed to serve smaller populations. Growth and insufficient financial resources for upgrades have caused many communities to discharge treated wastewater that does not comply with current Federal regulations for wastewater quality. Aquatic organisms in water bodies receiving municipal wastewater are often negatively impacted by exposure to these discharges. Exposures to contaminants of emerging concern (CECs) of wastewater origin have been associated with feminization of male fish and effects on the immune system of freshwater mussels. In some areas of the Grand River watershed (ON, Canada), there are abundant populations of freshwater mussels, including several species at risk (SAR). The impact of wastewater discharged into Boston Creek, a tributary of the Grand River, was investigated in partnership with a small rural community of approximately 300 people. During the fall discharge, the wastewater in the lagoon met regulations for wastewater quality and did not cause acute toxicity to Oncorhynchus mykiss. However, in static toxicity tests with the glochidia (larvae) of Lampsilis fascilola and Villosa iris, exposure to undiluted lagoon water reduced survival. During the fall discharge from the lagoon, adult mussels (Lasmigona costata) were caged upstream and downstream of the lagoon discharge, and tissues were analyzed for various biomarkers of contaminant exposure, such as oxidative stress and induction of metallothionein. Passive samplers were also deployed in the receiving waters to characterize exposure to CECs. Results will be presented and discussed with respect to the quality of the habitat of mussels, including SARs in the watershed.

**MP041 The independent and interactive effects of TBT and hypoxia on the oyster Crassostrea virginica**

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Oyster reefs provide significant ecosystem services, including shoreline stabilization, water quality improvement, and nursery habitat for commercially important fisheries. In Mississippi, historic oyster reefs have declined dramatically – from over 400,000 sacks in 2004 to less than 30,000 sacks in 2015. While overfishing has played a major role, other stressors such as environmental contaminants, hypoxia and disease, threaten the survival of oyster reefs in the Gulf of Mexico. The biocide tributyltin (TBT), once commonly used as an antifouling paint on ships, increases mollusk susceptibility to calcification anomalies, metal accumulation, predation, disease and imposex. TBT degrades slowly in the environment, potentially persisting for decades in sediment, where it may be subject to resuspension. Although banned worldwide in 2003, enforcement against TBT use is not universal. Expansion of the port at Gulfport risks reintroduction of TBT to the Mississippi coast. Additionally, most of the Northern Gulf of Mexico is referred to as a "dead zone" due to hypoxia caused by eutrophication from the Mississippi River. This study examines the effects of TBT and hypoxia utilizing a state-of-the-art toxicology facility at the University of Southern Mississippi's Gulf Coast Research Laboratory. Oysters in replicate exposure tanks are exposed to independent and combined hypoxia and TBT treatments in a factorial design: normoxic control (> 8 mg/L dissolved $O_2$), hypoxia (< 2 mg/L dissolved $O_2$), and diluted TBTCl (80 ng/L). Exposure time-dependent physiological and molecular endpoints, including food clearance rates and upregulation of metallothionein and calmodulin, will be compared with those from a field study of oysters deployed on historical oyster reef sites in the Mississippi Sound. The results of this study not only determine how these stressors affect oysters independently and in combination, but will provide valuable insight for decision-makers involved in the restoration and recovery of oyster reefs on the Mississippi coast. This project is paid for by the SETAC Student Training Exchange Opportunity and with Federal funding through the U. S. Department of the Treasury and the Mississippi Department of Environmental Quality under the Resources and Ecosystems Sustainability, Tourist Opportunities, and Revived Economies of the Gulf Coast States Act of 2012 (RESTORE Act).

**MP042 Harnessing lipidomics to characterize multiple stressor responses in zebrafish**

*D.A. Dreier, University of Florida / Physiological Sciences; M. Nouri, University of Florida; N.D. Denslow, C.J. Martyniuk, University of Florida / Physiological Sciences*

In the natural environment, aquatic organisms encounter multiple stressors including chemical contaminants and temperature gradients. Although most environmental risk assessment frameworks offer conservative thresholds for chemical stressors, there remains a need to characterize how additional stressors affect these criteria. This is especially important for chemicals affecting metabolism (i.e., mitochondrial function, lipid metabolism), as temperature can modulate these pathways. Thus, the objective of this research was to characterize the impact of temperature stress on mitochondrial-induced toxicity during zebrafish development. In addition, we aimed to determine how chemical-temperature stressors affect lipid composition during embryonic development, as altered lipid metabolism is a common response to mitochondrial dysfunction. To accomplish these objectives, we modified the fish embryo toxicity assay (OECD Test No. 236) to increase experimental throughput for multiple stressor exposures. Zebrafish embryos (n = 16/group) were exposed to the mitochondrial toxicant 2,4-dinitrophenol at 6 concentrations (0, 6, 12, 18, 24, 30 µM in 0.03% DMSO) and 2 temperatures (28, 33°C) for 96 hours. Embryos were monitored every 24 hours for lethality and hatch. It was determined that temperature significantly increased the rate of development, thus data were analyzed as a function of degree days, which provided normalization for hatch rate. At 28°C, there was an observable decrease in hatch success at 12 µM, and no fish hatched at higher concentrations (18, 24, 30 µM). However, at 33°C, there was greater hatch success at 12 µM, and hatch was also observed at 18 µM. Similarly, there was a decrease in mortality rate at and above 12 µM for zebrafish exposed to 33°C. These data suggest that temperature may offer a protective effect during early life stages, likely due to metabolic compensation and exposure duration at specific developmental stages. In addition to these findings, we have optimized a LC-MS/MS method to characterize global lipid responses in zebrafish larvae. In optimization experiments,

our method successfully quantified 679 lipid species including cholesterol esters, sphingomyelins, triacylglycerols, and several phospholipid classes, and we will use this approach to identify novel lipid responses that underlie mitochondrial dysfunction. Taken together, these data will be useful to further define adverse outcome pathways for multiple stressors.

**MP043 Toxicity of nano-enabled azoxystrobin on zebrafish embryo**

*Y. Zhang, G. Goss, University of Alberta / Biological Sciences*

The use of nanotechnology to enhance pesticide formulations holds the promise of reduced pesticide use, reduced mobility in soils and overall improvements in agricultural practices while simultaneously maintaining yields. However, the toxicity of nano-enabled pesticides, including azoxystrobin, has not been well studied comparing to their bulk form counterparts. This study investigates both lethal and sub-lethal endpoints in zebrafish embryos up to 120 hours post fertilization (hpf) under laboratory light or simulated sunlight. The median lethal concentration (LC50) of both nano-enabled and bulk form azoxystrobin are determined. Malformations, including pericardial edema, tail deformity, yolk sac edema, and spinal curvature are observed. The gene expression of stress-related genes, including *catalase, cyp1a, gst, sod1* and *sod2*, and enzyme activities of oxidative stress-related enzymes, such as Catalase, SOD, GSH and CYP1A, are measured and compared between nano and bulk azoxystrobin. The oxygen consumption of embryos is measured at 48, 72, 96 and 120 hpf. The preliminary results show that the LC50 of nano-enabled azoxystrobin is significantly lower than bulk azoxystrobin under laboratory light. In addition, the simulated sunlight significantly decrease LC50 of both nano-enabled and bulk azoxystrobin. The results will provide important information on the toxicity of nano-enabled azoxystrobin under ecologically realistic conditions.

**MP044 Distributions and potential ecological impacts of organic chemicals, metals, and metalloid in water and sediment from the Geum River Estuary, Korea**

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The Geum River Estuary (GRE) has been subjected to drastic environmental changes, including a dike construction and development of industrial complexes during the past 30 years. But, information on the spatiotemporal contaminations of seawater and sediment is not well known. In this study; i) distributions and sources of trace organic chemicals (TOrCs) including 24 polycyclic aromatic hydrocarbons (PAHs) and 6 alkylphenols (APs), ii) distributions of metals and metalloid, and iii) key environmental factors controlling the pelagic and benthic community structures were determined in GRE. A total of 113 seawater and 97 surface sediments were collected from the estuarine area in 2014 to 2015. Target PAHs and APs were detected in all sites and their concentrations significantly varied depending on the geological location. Although the concentrations of PAHs and APs were generally less than the suggested quality guidelines (Canada), great APs concentrations in seawater exceeding corresponding quality guidelines were detected in the inner estuary. Grass, biomass, and coal combustion were found to be the major PAHs sources and fresh input of octylphenols was identified, suggesting that industrial complexes continued to be the major sources of PAHs and APs in the given environment. The distributions of metals and metalloid were relatively high in the sediment of the inner estuary, while similar in the corresponding water column. The concentrations of metals and metalloid were generally less than corresponding guidelines (NOAA and CCME), but concentrations of some metals in sediment exceeded the Interim Sediment Quality Guidelines (ISQG, Canada). In aspect of community responses, TOrCs, metals, and metalloid in sediment, except mercury, were significantly correlated with the index of macrofaunal richness ($r$ = -0.34 -0.50, $p < 0.01$) in negative manner but with dominance ($r = 0.20$ 0.25, $p < 0.05$ or $r = 0.29$ 0.38, $p < 0.01$) of macrozoobenthos community in positive manner, respectively. These results indicated that lesser concentrations of TOrCs, metals, and metalloid could affect the community structure of macrozoobenthos. Whilst, there was no significant association with the plankton community. Overall, this research provides useful information about the contamination level, sources, and potential ecological impacts of water and sedimentary contaminants in the one representative estuarine area of the Geum River.

**MP045 Bioaccumulation Characteristics of Polycyclic Aromatic Hydrocarbons by *Mactra veneriformis* exposed to Oil-suspended Particulate Matter Aggregates**

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Dispersion and biodegradation of spilled oil are greatly enhanced by formation of oil-suspended particulate matter aggregates (OSAs), but little is known for impacts of OSAs on benthic invertebrates or microbial communities. In this study, followings were assessed: (1) bioaccumulation characteristics of polycyclic aromatic hydrocarbons (PAHs) by bivalvia, *Mactra veneriformis* and (2) relative abundances of microbes in sediments, during long-term feeding of OSAs. Concentrations of PAHs increased rapidly during the first week of OSAs exposure, peaked at Day 30, then declined until the end of the period of exposure. Patterns of PAHs bioaccumulation by *M. veneriformis* varied among PAHs compounds: two representative patterns were identified, one group for which bioaccumulation was constant, the other that rapidly increased up to peak then turned to decrease. Bioaccumulation of PAHs by *M. veneriformis* was dependent on changes in relative abundance of Gammaproteobacteria, reflecting active degradations of PAHs by selected genus. Four key genus included; *Porticoccus*, *Cycloclasticus*, *Alcanivorax*, and *Alkalimarinus*. These results are the first to demonstrate community-level interactions that can be considered and applicable to the use of OSAs for cleanup of spilled oil.

**MP046 Ecological Risk Assessment of Total Petroleum Hydrocarbon (TPH) Mixtures – ITRC Guidance**

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This presentation will provide an overview and decision-making process for addressing ecological risk from complex TPH mixtures that often function as both physical and chemical stressors to ecological receptors. The Interstate Technology and Regulatory Commission (ITRC) has developed a guidance document for risk evaluation of total petroleum hydrocarbons (TPH). While TPH are widely distributed in the environment, there is currently no comprehensive or systematic guidance at the national or state level to evaluate ecological risks associated with TPH. A systematic and comprehensive approach to identifying the TPH chemicals of potential ecological concern (COPECs) is presented in the guidance, by considering COPECs on the basis of origin, composition and environmental behavior. Sources and methods for development of screening values and toxicity reference values and several options for risk characterization are presented. A discussion of strengths, limitations and uncertainties for both mechanistic models and empirical data-based approaches will be presented with examples and case studies. This document will be the first comprehensive presentation of ecological risk assessment methods for TPH chemicals and will be released to the public in late 2018.

**MP047 Assessing the effects of environmentally relevant concentrations of antidepressant mixtures to fathead minnows exposed over a full life cycle**

*J. Parrott, Environment and Climate Change Canada / Aquatic Contaminants Research Division; C.D. Metcalfe, Trent University / Water Quality Centre*

Antidepressant drugs have been detected in municipal wastewater effluents (MWWEs) at ng/L to low µg/L concentrations. To assess the potential of these compounds to affect the survival, development and reproductive capacity of fish, we exposed fathead minnow (*Pimephales promelas*) over a full lifecycle in a flow-through system to a mixture of five antidepressants at nominal concentrations that have been detected in an Ontario MWWE; that is, venlafaxine at 2,400 ng/L, citalopram at 240 ng/L, fluoxetine at 90 ng/L, sertraline at 20 ng/L, and bupropion at 90 ng/L. In addition to exposure to this 1x AntiD Mix, we also exposed minnows to 10x concentrations of these MWWE antidepressants (i.e. 10x AntiD Mix). Mean measured concentrations of venlafaxine, citalopram, fluoxetine, sertraline, and bupropion were 2,300, 160, 110, 7 ng/L, and below detection limits, respectively, for the 5 compounds in the 1x AntiD Mix, and 33,000, 2,900, 1,000, 210, and 100 ng/L, respectively, for the compounds in the 10x AntiD Mix. During the full life-cycle exposure period of 167-168 d, no significant changes were observed in survival of fathead minnows. When male fish from the exposed treatments reached maturity, their somatic weights were increased and secondary sex characteristics were enhanced compared to control males. There were no significant differences relative to controls in condition factor, gonadosomatic index, or liver-somatic index in the exposed fish. Exposed fathead minnows produced similar numbers of eggs as control fish. There were no changes in nest-defense behaviours of male minnows that were exposed to the antidepressant mixtures. Egg quality, % fertilization, and % hatching in F1 fry were unaffected by exposure to the antidepressants. Eggs hatched 0.5 d earlier, deformities in fry were 50% lower, and there were transient decreases in length of F1 larvae at 8 dph in offspring from the treatment with the 10x AntiD Mix in comparison to controls. Overall, exposure to the antidepressant mixture at environmentally relevant concentrations (i.e. 1x AntiD Mix) caused no adverse effects in fathead minnows. Exposure to the 10x AntiD Mix increased the weight of adult male minnows and caused subtle effects in F1 offspring. This study is the first to assess sublethal effects in fish exposed to mixtures of antidepressants over a full lifecycle.

**MP048 Effects of imidacloprid to *Oreochromis niloticus* in mixtures with a pyrethroid or an organophosphate insecticide**

*P.B. Asanga Fai, Y.J. Tala Towa, University of Dschang / Department of Animal Biology*

Pyrethroid insecticides have been used for more than 20 years worldwide to control a variety of insect pests in different settings. Neonicotinoids are a new insecticide class which are important to agriculture because of their activity against sucking insects and some Heteroptera, Coleoptera and Lepidoptera. These pesticides have been detected in a variety of environmental samples and therefore there is significant concern about their potential toxic effects on non-target organisms. Interactions of components in a mixture can cause complex and substantial changes in the apparent properties of its constituents which may result in risk underestimations if only the individual toxicities are considered. We conducted this study to determine the effects of Imidacloprid and Cypermethrin mixtures on *Oreochromis niloticus* fingerlings. Mixture toxicity tests were carried out following a composite experimental design which combines aspects of the n·n design and Ray design for covering any possible interactions at various mixture ratios. Cypermethrin was found to be 6103 times more toxic than Imidacloprid. Our results also showed dose-dependent effects where at environmentally relevant concentrations greater than additive effects were observed while at higher concentrations, antagonism occurred. We conclude that concentration addition may not be an appropriate model for risk assessment of these mixtures as effects may be underestimated.

**MP049 Up a Creek Without a Fin: Applying Bioeffects Tools to Investigate Fish Fin Erosion in an Industrial Effluent Impacted Freshwater Stream**

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Fish surveys in Newton Creek, a small tributary of Lake Superior, identified abnormalities in resident fish in 2016-17. The primary observation of fin loss or fin erosion could not be readily attributed to known chemical agents. This tributary receives finished industrial effluent, which contributes a complex mixture of chemicals to the system. Bioeffects-based approaches were employed at the creek to elucidate perturbed biological pathways and potential causative chemicals. Caged fathead minnows (*Pimephales promelas*) were deployed for 14 days at two sites along the creek near the effluent source ($21^{st}$ St) and further downstream ($3^{rd}$ St), and at a nearby creek not receiving finished effluent (Faxon Creek). Following in situ exposure, phenotypic responses such as fin abnormalities were recorded and tissues (plasma, liver, gonad, fin clips) collected for biochemical and gene expression analysis. A composite water sampler was deployed in parallel with caged fish to collect an integrated sample representative of the full exposure period. Composite samples were used for chemical characterization (metals, PAHs, total hydrocarbons) and for biological characterization using a number of cell-based bioassays. Caged fish showed sex-specific mortality following 14 d exposure, with full female survival at all sites but male survival reduced by 17% at Faxon Creek and 42% at $3^{rd}$ St. No mortality was observed at the $21^{st}$ St site. Site specific fin erosion was noted, though not to the extent previously observed in resident fish, with the highest occurrence observed in females at $21^{st}$ St. Initial screening of site water using a multiplexed bioassay indicated potential perturbation of a number of biological pathways including aryl hydrocarbon receptor, estrogen receptor, peroxisome proliferator-activated receptors, retinoid X receptor, constitutive androstane receptor, and pregnane X receptor. Chemical and biological results will be discussed as they pertain to potential contributions to observed fin erosion in resident fish. *The contents of this abstract neither constitute, nor necessarily reflect, official USEPA policy.*

**MP050 Environmental exposure to an urban wastewater effluent: Effects on lipid metabolism of northern pike**

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Municipal effluents are composed of a complex mixture of biological and chemical substances that can represent toxicological risks for environmentally exposed fish. The Montreal Metropolitan Community (MMC) (QC, Canada) discharges on a daily basis about 2.5 million $m^3$ of primary treated wastewater in the St. Lawrence River. The MMC effluent is known to be one of the major sources of halogenated flame retardants (HFRs) in this vulnerable freshwater ecosystem. A recent study reported that exposure to this effluent can perturb the energy metabolism of the predatory northern pike (*Esox lucius*) via alteration of liver enzymes involved in fatty acid synthesis and oxidation. The present study aimed to investigate the effects of environmental exposure of pike to the MMC effluent on their lipid metabolism using a metabolomic approach targeting 175 lipid-related compounds. Concentrations of HFRs in liver of pike were used as marker of exposure to the MMC effluent. Results showed that lower concentrations of lysophosphatidylcholines (LysoPC) and higher concentrations of phosphatidylcholines (PC) were found in pike exposed to the effluent. Negative correlations were found in pike between the ratio of phospholipid classes (LysoPC/PC) and the sum of polybrominated diphenyl ethers (PBDEs). Transcriptional analyses of genes involved in the synthesis and remodeling of phospholipids including phospholipases and lysophosphatidylcholine acyltransferases (*LPCAT*) are underway to better understand the modes of action and effects of primary urban effluent exposure on lipid metabolism in fish.

**MP052 Generalized concentration addition mixtures model predicts glucocorticoid receptor activation by environmental full and partial agonists**

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Endocrine disrupting compounds are pervasive in surface and waste waters worldwide. We, and others, have detected complex mixtures of glucocorticoid receptor (GR) agonists using both chemical analysis and non-targeted effects-based methods in recent water quality surveys. Although the GR is ubiquitously expressed throughout tissues of aquatic species and has been implicated in growth, reproduction, metabolism, and osmoregulation, the way in which a mixture contributes to a single biological endpoint remains unknown. Therefore, we characterized environmentally relevant GR agonists using a CV1 cell line transcriptional activation assay. Cells were treated with individual GR ligands, a fixed ratio mixture of full and partial agonists, or using a two-chemical matrix design with full and partial agonists. Individual agonist efficacy (full vs. partial agonism) varied and potency ($EC_{50}$) ranged over several orders of magnitude, 48.09 to 102.5% and 1.278 x $10^{-10}$ to 3.93 x $10^{-8}$ M, respectfully. Concentration addition (CA) and response addition (RA) mixture models predicted concentration response curves within the observed 95% prediction interval bands ($EC_{50(OBS)}$ = 2.341e-10 M, 95% CI 2.114e-10 to 2.598e-10 M) for an equipotent mixture of 12 full agonists. However, for equipotent mixtures containing partial agonists (21-hydroxyprogesterone or corticosterone), the observed maximum efficacies were significantly lower than the CA and RA model-predicted maximum efficacies. Therefore, the generalized concentration addition (GCA) mixtures model, which accounts for agonists with < 100% reference agonist efficacy, was applied to predict non-equipotent mixtures containing full and partial GR agonists. The GCA modeled responses of matrix exposures for partial agonist + full agonist, and full agonist + full agonist, mixtures were identified as part of the same distribution as observed responses ($p < 0.05$). The GCA model resulted in the best overall fit for mixtures containing both full and/or partial GR agonists compared to the models without efficacy variables. Elucidating the mechanistic basis of GR activation by environmental ligands will not only aid in characterizing environmental mixture samples using this effects-based approach, but will also provide a foundation for future studies in predicting and interpreting potential phenotypic adverse outcomes when the approach is applied as a chemical screening tool. *Abstract does not necessarily reflect USEPA views or policy.*

**MP053 Effects-based monitoring of bioactive contaminants associated with exposure to wastewater treatment plant discharge on the Colorado River**

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Over the last several years, chemical monitoring of the Colorado River near Moab, UT, has detected a variety of chemicals of emerging concern (CECs). However, given the lack of available effects data for most of the compounds detected, potential ecological risks, if any, associated with these contaminants are unclear. To help address this question, extracts of surface water samples were analyzed using a multiplexed in vitro bioassay that evaluated activity with regard to human nuclear receptors and related transcription factors. Estrogen receptor (ER), glucocorticoid receptor (GR), and peroxisome proliferator activated receptor (PPAR) activities were associated with surface water near the Moab wastewater treatment plant (WWTP). In vitro ER activation was also confirmed in surface water near the WWTP outflow using the estrogen-responsive T47D-Kbluc bioassay. To evaluate the potential bioavailability and significance of these biologically-active contaminants, adult fathead minnows (*Pimephales promelas*) were caged at five sites along the Colorado River near Moab, UT, at various proximities to the WWTP. After four days of exposure, skin mucus, plasma, livers, and gonads were collected for measurement of a variety of biological endpoints (e.g., transcriptomics, metabolomics, steroid concentrations). An autosampler was simultaneously deployed to collect a composited surface water sample reflective of the four-day fish exposure. Composite water samples were analyzed for a suite of pharmaceuticals, pesticides, steroid hormones, wastewater indicators, and in vitro biological activities (e.g., ER, GR, and PPAR activities). Consistent with previous monitoring, total estrogenic activity of surface water was confirmed using the T47D-Kbluc bioassay, with approximately 45 ng/L of 17β-estradiol equivalents (E2-EQ) estimated at the WWTP discharge site and 2 ng/L E2-EQ at the proximal downstream site. Consistent with downstream dilution of estrogenic activity, no statistically significant differences were observed in male liver vitellogenin mRNA expression among any of the sites, excluding the WWTP site in which 100% mortality was observed. Results suggest rapid dilution of biologically-active contaminants downstream of the WWTP discharge and will be discussed relative to the potential effects of CECs in the Colorado River. *The contents of this abstract neither constitute nor reflect USEPA policy.*

**MP054 The Enigmatic Disappearance of the Once-common Leopard Frogs from Long Island: Are contaminants to blame?**

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Globally, amphibians are in decline, and in some populations, declines have been attributed to the presence of pesticides and other contaminants in the environment. Relatively few toxicological studies focus on bioaccumulation of such contaminants in amphibian tissue, likely because such studies on amphibians are not required by the federal government. However, knowledge of the bioaccumulation of these toxicants could inform local agencies and concerned citizens as to which toxicants require additional regulation and restriction to protect threatened and endangered amphibian populations. We hypothesized that profiles of bioaccumulated pesticides and other non-metallic contaminants would differ between a site where leopard frogs are present in central New Jersey (NJ) and other sites where leopard frogs are now extirpated on Long Island (LI), New

York. The objectives of this study were to determine (1) detectable toxicant levels in amphibian tissues sampled from NJ and LI, and (2) whether the extirpation of leopard frogs from LI can be explained by differences in contaminant detection between rural parts of LI and NJ. We examined southern leopard frog tadpoles (Rana sphenocephala) from NJ and a combination of enclosure-raised southern leopard frog tadpoles and wild-collected green frog (*Rana clamitans*) and bullfrog (*Rana catesbeiana*) tadpoles from LI. Tadpoles were homogenized in groups for each replicate sampling. Gas chromatography/mass spectrometry (GCMS) was used to detect the 73 most widely detected and distributed contaminants in New Jersey and New York regions of the United States, and a PCA analysis was used to determine the most predictive contaminants. We found that many chemicals commonly detected in freshwater aquatic habitats were not present at detectable levels in our samples. Polycyclic aromatic hydrocarbons were the most prevalent class of toxicants found in all frog tissue samples and were statistically significant in the PCA analysis. All frog tissue samples contained fluoranthene, PCB52, and PCB118. Although sites were similar in their chemical profiles, the leopard frog site had lower levels of DDE, DDD and phenanathrene when compared to non-leopard frog sites. Pesticides alone do not explain the absence of leopard frogs from sites in LI. Future studies should compare a larger number of habitats that continue to support the leopard frog and habitats from which this species has been extirpated.

### MP055 Bioaccumulation of butyltins in marine environment and their impacts on intertidal benthic community

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Thirteen years ago, the Korean Government introduced a regulation prohibiting the use of tributyltin (TBT), which was a component of antifouling paints. A subsequent decline in the concentrations of butyltins (BTs) was recorded in seawater and sediment, however, the current concentrations of BTs in biota have not been well documented. The spatiotemporal distributions and concentrations of BTs were recorded in biota from 2013 to 2015 along the coasts of Samcheok and Tongyeong using GS/MSD analysis. Crustaceans contained the greatest concentrations of BTs, followed by gastropods, fishes, and bivalves. We found that the concentrations of BTs were relatively greater at Tongyeong compared to those in Samcheok, primarily because of the geographical characteristics of the area. We also confirmed that the regulation has been effective by showing that the TBT concentration decreased over the 3-year study period. The TBT concentrations of gastropods and bivalves fell within the limits of the guidelines and/or the effective concentration of the toxicological endpoint reported previously. The concentrations of BTs also varied among species, being dependent on the weight of the soft tissue. Furthermore, the greater quantities of BTs degradation products compared to TBT confirmed the absence of recent inputs of the very pollutants during the study periods. We investigated the benthic community in the study area for estimating butyltins' impacts on the benthic community. We found butyltins degradation index was significantly related with several ecological indices ($p < 0.05$). However, compared to other Asian countries, biota BTs were greater in Korea, with noticeably greater concentrations along the south coast. Thus, further investigation of the distributions of BTs along the Korean coasts would be timely required. In conclusion, our results provide useful background information about the recent trends of BTs in Korea.

### MP056 *Microcystis aeruginosa* adversely impacts *Daphnia* spp.: Implications on food webs in the Great Lakes

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Harmful algal blooms dominated by *Microcystis* are a troublesome nuisance to freshwater ecosystems used for drinking, irrigation, fishing, and recreational purposes. Many *Microcystis* blooms produce strains of the hepatotoxin microcystin that is capable of inflicting harm to zooplankton. Research on the effect of microcystins on zooplankton remains elusive; from one perspective, studies suggest *Daphnia* can be used to suppress phytoplankton, including cyanobacteria, and coexist in toxic blooms; however, studies have also indicated toxic effects on *Daphnia* that consume cell-bound microcystin. This dual perspective may be attributable to an evolutionary adaptation in *Daphnia* that ensures survival and reproduction in toxic cyanobacterial blooms. We examined reproduction and survival of laboratory-cultured *Ceriodaphnia dubia* and *Daphnia magna* in microcystin-producing *Microcystis aeruginosa* through a series of life-cycle bioassays. Test organisms were exposed to a concentration gradient ranging from 0.5 µg/L to 100 µg/L microcystin-LR which corresponds to values typically found in the Great Lakes during bloom season. Mortality was observed in *C. dubia* ($LC_{50}$ = 2 µg/L) and *D. magna* ($LC_{50}$ = 37 µg/L) exposed to microcystin-LR, and reproductive effects at concentrations as low as 2.5 µg/L. This information will improve our understanding of the risks posed to food webs in the Great Lakes.

### MP058 Acute sub-lethal and lethal toxicities of elevated $CO_2$ on various stages of marine medaka *Oryzias melastigma*

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The potential leakage from marine $CO_2$ storage sites is of increasing concern, but few studies have evaluated the probable adverse effects on marine organisms. Fish, one of the top predators in marine environments, should be an essential representative species used for water column toxicity testing in response to waterborne $CO_2$ exposure. In the present study, we conducted fish life cycle toxicity tests to fully elucidate $CO_2$ toxicity mechanism effects. We tested sub-lethal and lethal toxicities of elevated $CO_2$ concentrations on marine medaka (*Oryzias melastigma*) at different developmental stages. At each developmental stage, the test species was exposed to varying concentrations of gaseous $CO_2$ (control air, 5%, 10%, 20%, and 30%), with 96 h of exposure at 0–4 d (early stage), 4–8 d (middle stage), and 8–12 d (late stage). Sub-lethal and lethal effects, including early developmental delays, cardiac edema, tail abnormalities, abnormal pigmentation, and mortality were monitored daily during the 14 d exposure period. At the embryonic stage, significant sub-lethal and lethal effects were observed at pH < 6.30. Hypercapnia can cause long-term and/or delayed developmental embryonic problems, even after transfer back to clean seawater. At fish juvenile and adult stages, significant mortality was observed at pH < 5.70, indicating elevated $CO_2$ exposure might cause various adverse effects, even during short-term exposure periods. It should be noted the early embryonic stage was found more sensitive to $CO_2$ exposure than other developmental stages of the fish life cycle. Overall, the present study provided baseline information for potential adverse effects of high $CO_2$ concentration exposure on fish developmental processes at different life cycle stages in marine ecosystems.

**MP059 Development of a juvenile fathead minnow fin regeneration assay for use in evaluating chemical involvement in fin regeneration impairment**

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Teleost fish are among the few vertebrates with the ability to nearly perfectly regenerate an amputated or damaged limb (i.e., fins). Fin damage can result from a wide range of natural sources including abrasive habitat surfaces, predation, conspecific aggression, and nutritional deficiencies. However, increased incidence of fin erosion has also been observed at sites impacted by a wide range of pollutants including pulp mill effluents, coal-ash runoff, oil sands process waters, etc. This suggests that exposure to certain chemical contaminants may impair the processes that support fin regeneration and repair. Development of a laboratory-based fin regeneration assay would have utility for both testing this hypothesis, as well as screening of both individual chemicals and complex mixtures for their ability to impair fin regeneration processes. The present study reports on the time-course of fin regeneration in 30 d posthatch fathead minnows (*Pimephales promelas*) following partial caudal fin amputation. Fish were sampled daily from 0-7 days post amputation (dpa). Fish length, caudal fin area, and specific fin ray lengths were measured at each time point using image analysis. Additionally, RNA was extracted from caudal fin samples and expression of several genes known to play a role in fin regeneration, including fibroblast growth factor receptor 1a (fgfr1a), lysyl hydroxylase (lhl1) and patched-1 (ptch1), was evaluated using quantitative polymerase chain reaction (qPCR). Based on averaged fin ray length and/or fin area as determinants, statistically significant regrowth was detectable by 4-5 dpa (25° C). Both ptch1 and lhl1 expression was significantly increased at 3 dpa compared to unclipped controls, suggesting these as potential molecular markers of the initiation of fin regrowth. A statistical power analysis focused on the phenotypic measurements of fin regrowth suggest that a sample size of n=10 measured at 7 dpa would likely be statistically robust for detecting treatment effects on regeneration. Results inform development of a standardized test that can be used for routine screening. *The contents of this abstract neither constitute, nor necessarily reflect, USEPA policy.*

## Deep Learning for Predictive Toxicology

**MP060 QSAR Modeling for Predicting the Inhibitory Concentration (IC50) of Organophosphates for Human Acetylcholinesterase**

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Organophosphates (OP) are broad-spectrum insecticide and nerve agents which is majorly used in agriculture in India. OP is diverse category of molecules with varying functional groups which contribute their physical and chemical properties. Most of our knowledge about OP and their toxicity or pharmacodynamics/pharmacokinetics is based upon laboratory animals. Therefore, when it comes to combat OP poisoning in human, we are forced to rely upon these research data. In this research we have developed a QSAR model which could predict the inhibitory activity ($IC_{50}$) of OP against human AChE. The tool used for QSAR model was Easy QSAR 1.0 and activity chosen was $IC_{50}$ of OP against human AChE. Molecular weight (weight), H-bond acceptor (HbondAccp), Gutman molecular topological index based on valence vertex degree(log10) (GMVTIV), logP, number of ratable bonds (nrot), number of aromatic bonds (naro), topological polar surface area (TPSA) and weiner Index(W) are the eight descriptors used to build the models. Four sorting methods were used namely: random, ascending order of inhibitory activity, ascending order of molecular weight and ascending order of TPSA and each sorted list was divided by three methods: "50-50", "Alternate and 50-50" and 60-40. Each training set was validated before validating the test set. The best QSAR model obtained was having $R^2$ of 96.9 and Adj. $R^2$ was 96.4 (test set validation) with combination of sorted list "ascending order of TPSA" and division 60-40. The resulting model has $R^2$ 90.4 and Adj. $R^2$ 89.5 while validating the training set. This model has -0.53 of bias and 0.63 RMSD. All statistical calculation was done using Minitab and MS excel. The study infers that a QSAR model has been developed which can efficiently predict the $IC_{50}$ of the OP compounds for human AChE.

**MP061 The effects of ionizing radiation on mutation rates: A meta-analysis**

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The effects of low-dose ionizing radiation from environmental contamination on mutation rates vary throughout the literature. A comprehensive view of these effects have yet to be determined, as studies vary greatly in their experimental design, level and type of contamination, and the organisms on which effects are tested. To characterize when ionizing radiation causes an increase in mutation rate, we conducted a meta-analysis of the published literature. We classified studies based on the methods of detecting mutation rates (or endpoint), source of radiation contamination, and taxonomic group. The effects were determined for each study by extracting and converting statistics to the same effect size, Fisher's transformed Pearson correlation (Zr). We included 112 studies, with a total of 282 estimates of effect. Overall, data show an increase in mutation rates due to ionizing radiation. An analysis of variance showed that extensive variation existed among studies according to their endpoint used, type of radiation contamination, and taxonomic group. Large effect sizes were observed in studies of chromosomal aberrations, following nuclear accidents, and in organisms other than mammals. Our findings point to chromosomal aberration as a sensitive method for detection of changes in mutation rate induced by ionizing radiation, and possibly other environmental mutagens. The analysis of the effects of ionizing radiation plays a large role in understanding and mitigating damages following radioactive contamination. It is also useful in understanding natural variation in susceptibility to ionizing radiation and other mutagens, under different contamination scenarios.

**MP062 Quantitative Target-specific Toxicity Prediction Modeling: A Novel Predictive Toxicology Approach**

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Quantitative structure-activity relationship (QSAR) modelling is a chemical descriptors-based approach for quantitative prediction of biological activity, potency or toxicity of a chemical. QSAR modelling may suffer low prediction accuracy in the absence of information on chemical-biomacromolecule interactions. In order to mitigate this problem, we developed a novel Quantitative Target-specific Toxicity Prediction Modeling (QTTPM) approach that integrated molecular dynamics (MD) simulation and machine learning. As a proof-of-concept study, we chose androgen receptor (AR) as the toxicant-targeted biomacromolecule because AR is a nuclear receptor playing crucial roles in the development of male reproductive system and tumors in prostate, bladder, liver, kidney and lung. Molecular docking and MD simulations were employed to generate a new set of dynamic protein-ligand interaction descriptors (dyPLIDs) used for developing QTTPMs. We selected 274 chemicals (154 agonists/120 antagonists) with quantitative AR assay outcomes from Tox21 datasets. First, we performed five 100-ns MD simulations of AR crystal structures in its un-bound (*apo*), two agonist-bound (testosterone and dihydrotestostrone), and two antagonist-bound (R-bicalutamide and cyproterone acetate) forms and identified key interaction patterns leading to >400 dyPLIDs. Second, 6-ns MD simulations of 274 AR-ligand docked complexes were performed to calculate dyPLIDs. Third, Random Forest (RF) algorithm was deployed to identify key descriptors (including both

conventional 1D/2D/3D descriptors and dyPLIDs). Fourth, QTTPMs were built using the key descriptors and AR assay data. QTTPMs demonstrated superior accuracy than QSAR models constructed with conventional chemical descriptors. In addition, QTTPMs provided insights of key protein structural changes upon ligand binding that modulated the activity of the AR. The novel QTTPM approach was developed using a small dataset of 274 AR agonists/antagonists. Although more biomacromolecular targets and chemicals warrant further investigations, this study demonstrates the superiority of QTTPM over QSAR and that QTTPM is a promising new tool for computational predictive toxicology.

**MP063 Predicting Fish Embryo Development in Videos using Convolutional Encoder and Decoder Neural Network**

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Embryo development follows a set of defined spatial and temporal patterns. If deep neural networks (DNNs) can learn those patterns from a collection of normal embryo development videos then they could predict how embryo will develop in the next few video frames (near future). Conventional DNNs have been used to learn from the past toxicology studies (training sets) and to predict possible adverse outcomes. Those approaches rely on supervised learning in which each record of the training data has a manually-assigned label that indicates the outcome of each study (i.e., toxic/non-toxic or the degree of toxicity). Because each video frame in the training videos contains large number of pixels it is not feasible to assign one label to each pixel. Hence, here we propose a novel, semi-supervised learning approach. The semi-supervised DNN trained in this study uses an encoder-decoder architecture. The encoder part of our DNN learns to compress each input video frame $F_t$ at time t into a low-dimension vector $V_t$ such that it can be used by the decoder to re-construct each video frame $F_{t+1}$ at time t+1. More specifically, the decoder learns to decompress the vector $V_t$ into an image that is as close as possible to the next video frame $F_{t+1}$. In this semi-supervised learning process, the next video frame $F_{t+1}$ is used as the learning target (outcome) for each previous video frame $F_t$, avoiding the need of assigning labels to all pixels in all video frames. A sequence of convolution layers was used in our DNN encoder to perform the convolutional operations that extract subtle patterns embedded in each video frame. Another sequence of transposed convolution layers was used in our DNN decoder to re-construct/predict the next video frame. Original and predicted embryo development outcomes (over a 6 h period) were generated and compared. The results demonstrate that our DNN is capable of closely predicting embryo morphology in the next video frame. The left side of each sub-figure in Figure 1 at https://drive.google.com/open?id=1TRAqNUHpFApQ-pLmIZNTwCHvhYFwZj7L shows the original video frame at different time over a 6-hour period in a video showing zebra fish embryo development. The right side of each sub-figure in Figure 1 shows the predicted embryo development generated from our convolutional encoder-decoder DNN. The results demonstrate that our DNN is capable of closely predict the embryo's new structure in the next video frame.

**MP064 Graph wavelet neural network for toxicity prediction**

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Quantitative structure activity relationship(QSAR) methods have been widely used in the toxicity prediction of organic molecules. Compared to traditional animal tests, QSAR methods are less time and cost consuming. In this study, we have developed random walk based graph wavelet scattering neural network for toxicity prediction. Graph wavelet was developed from molecule graph and was used to generate new molecule features from eight existing characteristics of molecules: atom total charges, atom valence charges, and one-hot encoding of: electron acceptor, electron donor, aromatic atoms, sp1, sp2 and sp3 hybridization. Our model was invariant to molecule permutation, translation and rotation. Multi-task learning was used to leverage additional information from different tasks. A three layer of neural network was built for training the new features and L1 regularization was used to acquire a sparse neural network and avoid overfitting. Multiple toxicity datasets were acquired from PubChem. Due to the imbalance distribution of data points among different classes in each dataset, we applied data augmentation by adding random noises to existing data points to create a larger database for neural network training. In each task, we combined 80% of data points from each category for training, 10% from each category for validation and 10% for test. Our model achieved state-of-art test accuracy on multiple datasets. The results indicated that our graph wavelet neural network models can be successfully used for toxicity predictions.

**MP065 Predicting Potential Adverse Outcome Pathways using Long-Short Term Memory (LSTM) Neural Network**

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Adverse outcome pathways (AOPs) organize relationships among initial chemical-biological interactions, intermediary key events, and adverse outcomes (AOs) relevant to risk assessment. AOPs have potential to support and enhance the use of mechanistic data in regulatory decision-making, but efficient ways of AOP development are lacking. Here we explore potential of training deep neural networks (DNNs) to absorb complex knowledge from large toxicology databases, and to predict potential AOPs. Because each AOP contains a sequence of causal entities, the task of predicting AOPs requires a special type of DNNs that can learn such sequentially causative relationships. For this study, a type of DNN known as the *Long-Short Term Memory* (LSTM) neural network was adapted and utilized. In this approach the prediction depends on, not just the most recent input, but also the learned weights of all the previous inputs. This LSTM capability is extremely useful in assessing the importance of causative relationships among previous AOP entities for predicting the next causative event or adverse outcome. While the LSTM architecture was designed to capture the relationships among sequential inputs, extensive training is essential for LSTM to grasp the causative relationships among so many AOP entities. We collected hundreds of millions of relationships among millions of concepts (including genes, chemicals, proteins, diseases) from various sources like Comparative Toxicogenomics Database (CTD), ToxCast Dashboard, Unified Medical Language Systems, 16-million abstracts of PubMed papers, and AOP Wiki. Since all the relationships were text in the data sources, they were first translated into 300-dimension vectors by another word-embedding neural network before inputting to our LSTM for training. To demonstrate the utility of above approach we first examined two sample predictions generated from our LSTM that was trained by using pairs of 104,194 relationships extracted from the CTD database. For each sample, when user presented a causal relationships between a chemical and an impacted gene, our trained LSTM predicted the top two possible AOs. Our results shows that the predicted AOs were very similar (i.e. drug induced liver injury) to the AOs recorded in the CTD database (i.e. fatty liver). Users can also present relationships between gene-disease or disease-chemical to our LSTM which can then predict chemical or gene, respectively.

## Bridging the Divide Between Research Scientists and Decision-Makers in Environmental Fate and Exposure Modeling

**MP066 Development of a global environmental exposure modeling framework for risk assessment of chemicals disposed down the drain: A case study for China**

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Environmental exposure assessment of chemicals that are disposed down the drain (such as consumer product ingredients) at the global scale within a consistent and accessible framework has remained a challenge over the years, despite advancements in exposure modeling and global and local data resources. Historically, assessment efforts have been tailored and applied to specific geographies and used simplistic approaches rather than to build a spatially resolved global assessment infrastructure. Challenges such as inconsistent, scarce, or rapidly-evolving data resources, particularly for developing countries where assessment needs are high, have further complicated the evolution of spatially resolved global exposure assessment tools. However, through strategic integration of existing global data resources and established modeling tools, a standardized framework and methodology for GIS-based exposure modeling can be developed for the global scale. In this study, we present a spatially resolved global environmental exposure model approach designed to incorporate best-available data and modeling tools, using China as a case study. The global hydrology network from HydroSHEDS and HydroBASINS (Lehner et al. 2008 and 2013), global river flow and population estimates, and best-available country-specific water use and wastewater treatment information were integrated with the GIS-ROUT exposure model (Wang et al. 2005) and iSTREEM® model framework (American Cleaning Institute) to provide a means of estimating the distribution of concentrations of a chemical disposed down the drain across a river network based on chemical production volume and consumer usage estimates. Both wastewater treatment plant effluent and direct discharge are accounted for by the model through estimation of catchment-specific emissions. The spatial nature of the model provides a robust means for estimating variability in environmental exposures. Details of the various model components and generated output for China are overviewed, as well as considerations and discussion regarding on-going extrapolation to the global scale. The framework developed as part of this model is highly adaptable to countries with an abundance of data (e.g., North America, Western Europe, etc.) or those scarce with data (e.g., developing countries) available to parametrize the model.

**MP067 Development of a spatially resolved global mean annual flow dataset for use in environmental risk assessment: A case study for China**

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Environmental exposure models for chemicals used widely across large geographic areas and disposed of down the drain are important tools for informing ecological risk assessments. One important element of these models is understanding the dilution of wastewater treatment plant (WWTP) effluent into the receiving stream (dilution factors) which allows for the estimation of in-stream environmental concentrations based on either estimated flow of receiving waters. In the U.S., the iSTREEM model (American Cleaning Institute) estimates dilution of WWTP effluent into receiving streams through the incorporation of a spatial hydrologic network with associated flow data (National Hydrography Dataset Plus) into the exposure model to spatially associate (and route) local WWTP emissions with corresponding local flows. A similar approach for generating localized dilution factors can be employed on the global scale to integrate the chemical emissions component of the model with a hydrologically-connected global river network with associated flow values. The HydroSHEDS and HydroBASINS datasets (Lehner et al. 2008 and 2013) provide a global hydrology dataset that can be used as a spatial hydrologic framework, including a network of streams and rivers and watershed and catchment boundaries. However, flow estimates corresponding to the global river network are a critical attribute that must still be incorporated for exposure modeling. Using China as a case study, a mean annual flow dataset to correspond with the HydroSHEDS and HydroBASINS global data was developed using the well-established Curve Number (CN) approach developed by Natural Resources Conservation Service (NRCS, USDA). The CN approach integrates environmental and landscape features including best available and high-resolution precipitation, soils, and land use characteristics to estimate surface runoff over the land area. The high-resolution runoff grid was spatially combined with hydrology datasets to derive flow estimates across a river network. Global datasets were utilized for model parameters so that the approach could be extrapolated to the global scale, while also providing the flexibility to incorporate best-available data. This presentation will provide a detailed overview of the runoff methodology, validation against measured flow data, and the resulting river flow dataset for China.

**MP068 A new tool for the toolbox: Predicting multi-pathway emission and fate of contaminants entering freshwater systems in Europe**

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Exposure models help to prospectively assess the potential for ecological exposures from releases of substances into the environment. Availability of newer data, increasing computing power and improved methods provide continuing opportunity to improve our ability to predict environmental exposures through models and add to our “toolbox”. We present a new model designed to encompass multi-pathway environmental emissions coupled with environmental fate components, contained in a modular and transparent framework which is scalable and portable to multiple geographies. This spatially-explicit model (presented here for Europe) is based on publicly available datasets, combined with a hydrologic framework containing geographically variable emissions linked to a river network simulating environmental transport via surface water. The hydrologic framework is based on a set of basins and rivers (WWF HydroSHEDs) linked to emission characteristics for each sub-basin (more than 37,000 in the EU-30). Emissions characteristics are derived from point-source wastewater data (EEA Waterbase) as well as diffuse source inputs, accounting for the potential of urban storm water runoff or other overland flow constituents. Concentrations of contaminants are routed through the river network based on local river attributes combined with assumptions about chemical fate in the aquatic environment. Multi-year, high-resolution data on river flow (FLO1K) are leveraged for an expanded set of possible modeling scenarios. Transparency is critical for model understanding and acceptance. Model documentation follows standard documentation protocol proposed by the European Committee for Standardization (CEN) as described in the 2016 CEN workshop: “*Promoting the acceptance and use of chemical exposure models through transparent documentation*”. Several scenarios will be presented covering different use/emission situations and substance fate characteristics, including the relative importance of different emission pathways (e.g., down-the-drain, urban storm water, land-based diffuse runoff) and environmental media. While the presented material is an example of environmental emission and fate of different substances, it represents a working framework implemented for Europe with viable application to other geographies.

**MP069 EPI Web Suite: Design features and enhancements for an online platform**

*W. Lee, EPA / OPPT; M. Kawa, SRC Inc. / Environmental Health Analysis; L. Cassidy, SRC Inc. / EHA; W. Meylan, SRC, Inc.*

The EPI (Estimation Programs Interface) Suite™ is a collection of physical-chemical property and environmental fate estimation programs maintained by the US Environmental Protection Agency’s (EPA) Office of Pollution Prevention and Toxics (OPPT). OPPT performs, and is responsible for, chemical hazard and risk evaluations under the Toxic Substances Control Act (TSCA), amended by the 2016 Frank R. Lautenberg Chemical Safety for the 21st Century Act. OPPT has developed estimation tools from extensive experience assessing chemicals under TSCA, including (quantitative) structure-activity relationships or (Q)SARs in EPI Suite™. Understanding the estimation methods and their appropriate application is important when running and interpreting model results. The underlying predictive methods and equations in EPI Suite™ have been published in peer-reviewed technical journals and are available in help files; however, it is also useful to convey model information to users while running the estimates in the context of a specific chemical. This presentation will discuss developments made to EPI Suite™, as a web-based platform, to increase transparency and present level of confidence information for the underlying methodologies to users. Specifically, features to facilitate interpretation of the model domain for each model's training and validation set will be highlighted. This includes an approach for displaying the chemical structures and empirical data for structural analogs using the structural fragment based recognition approach from the EPA's Analog Identification Methodology (AIM) Tool. Additionally, a demonstration of the features of the web based tool will be performed. *Disclaimer: The views expressed in this abstract are those of the authors and do not represent Agency policy or endorsement.*

**MP070 Bioavailability of Mercury in Power Plant Wastewater and Ambient River Samples: Evidence that the Regulation of Total Mercury is Not Appropriate**

*R.J. Reash, American Electric Power*

Mercury (Hg) is a neurotoxin that can cause debilitating effects to human and environmental receptors under high exposure conditions. For industrial and municipal point sources that discharge Hg, wastewater limitations on total mercury (THg) concentrations or loads are typical. While this regulatory practice provides simplicity for regulated industry and water resource agencies (re: analytical detection and reporting purposes), it ignores the important considerations of speciation and bioavailability. In this study water samples were collected from multiple power plant wastewater, simulated mixing zone, and ambient river locations (N = 10 to 20) and analyzed for bioavailable Hg (methylmercury and acid-labile mercury, or BHg) forms, THg, and dissolved Hg. The median concentration of THg in wastewater, mixing zone, and ambient river samples was 7.1, 5.3, and 2.3 ng/L, respectively. The percentage of THg as BHg (median values) were 18.7, 29.3, and 8.5% for wastewater, mixing zone, and ambient river samples. The percentages of methylmercury (MeHg) as THg were not statistically different between paired ambient and mixing zone samples ($P > 0.05$), indicating that wastewater did not increase the MeHg fraction when mixed with ambient water. Multiple regression analysis indicated that variation in THg for combined wastewater and mixing zone samples could be adequately explained by pooled water quality parameters (total suspended solids, total dissolved solids, sulfate, total organic carbon, pH, specific conductivity; $r^2 = 0.51$; $p < 0.05$), however no significant regression relationships were apparent for the percent of BHg. These results, at least for the wastewater samples evaluated, indicate that regulating THg is likely overly-conservative and mechanisms to regulate the bioavailable forms of Hg are needed. If Hg fish tissue monitoring data indicate concentrations are less than consumption thresholds, metal translator methodologies or bioavailability-based criterion techniques (as currently used for non-Hg trace elements) should be allowed for Hg.

**MP071 Chemical Hazard Screening Using GHS**

*S. Risotto, American Chemistry Council / CPTD; S. Dubrow, American Chemistry Council / Sustainability & Market Outreach; B. Howard, American Chemistry Council / Sustainability and Market Outreach*

Demand for information on the health and environmental hazards of chemicals has increased in recent years, as have calls for a transparent presentation of these data. These requests for more and more data have produced a need for sources of publicly available data and methodologies to translate that information into a form that can be readily understood and can be combined with other factors for alternative assessment. This need is particularly acute for small- and medium-sized any companies that do not have the resources to conduct extensive reviews of the scientific literature. Fortunately, the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) provides a globally consistent platform for reporting human and environmental hazard information that is the basis for many of the commercial hazard screening tools. The European Chemicals Agency (ECHA) has compiled this information into an easy-to-use search tool for the vast amount of GHS information that has been submitted as part of the European Regulation on Registration, Evaluation, Authorization and Restriction of Chemicals (REACH). The ECHA site provides GHS classifications for the 20,000+ REACH-registered chemicals. In most cases, the ECHA will provide all of the GHS information submitted by REACH registrants, as well as the number of entities that provided any particular set of GHS classifications. While GHS does not include classification for persistence and bioaccumulation, Environment and Climate Change Canada (ECCC) has generated a comprehensive evaluation of the P & B characteristics of the chemicals on their Domestic Substances List (DSL). That information is available from the ECCC website. As part of its Sustainability and Market Outreach initiative, the American Chemistry Council (ACC) has collaborated with several partners to collect the GHS and P/B classifications available from ECHA and ECCC and to translate that information into a well-known and respected hazard scoring system. Such a screening device for product designers and other non-toxicologists can help to demystify the hazard assessment process and complement existing efforts to evaluate multiple criteria, including hazard, in making chemical selection decisions.

**MP072 A Tiered Approach to Chemical Screening**

*S. Dubrow, American Chemistry Council / Sustainability & Market Outreach; S. Risotto, American Chemistry Council; B. Howard, American Chemistry Council / Sustainability and Market Outreach*

In today's age of increased inquiry into the chemistry of everyday products, how do Retailers and Brands respond to requests for more rigorous product safety assessments? There are a number of Alternatives Assessment (AA) frameworks used in the marketplace today to evaluate potential substitutions for chemicals in products. Most rely on chemical lists or scoring methodologies that are based solely on a chemical's inherent hazard to screen products and alternatives. However, risk is a function of hazard; consequence; and the likelihood the consequence will occur. For hazards presented by chemicals in products, the likelihood of consequence is often dependent on whether the chemical can be released from the product, and an individual subsequently exposed to that release. A more comprehensive risk assessment generally requires more extensive data input, and can involve technical models that are complicated to use without the needed education and training. To address this issue, ACC's Sustainability and Market Outreach division developed a tiered approach to chemical screening that provides a simple, yet science-based method for evaluating the potential for chemical exposure from a product. This more holistic framework for alternatives assessment can be used to introduce the concept of risk and exposure into existing hazard-only methodologies. In addition to an initial hazard screening, this approach includes higher-level tiers to assess whether a release is likely based on chemical and product characteristics. The third tier provides a risk scoring methodology based on the intended use of the product, and the final output is a risk matrix that can be used to prioritize actions and resources towards the highest risk scenarios. ACC also recently began a project to automate this tiered approach, to facilitate understanding and use of the scoring methodology.

**MP073 A Multi-Criteria Approach to Alternatives Assessment using multi-criteria decision analysis (MCDA)**

*B. Howard, S. Dubrow, American Chemistry Council / Sustainability & Market Outreach; S. Risotto, American Chemistry Council / Chemical Products and Technology Division*

When considering alternative chemicals in product formulations, how do you consider tradeoffs between disparate factors such as performance, cost, and lifecycle impacts? Multi-criteria decision analysis (MCDA) offers an established approach for well-informed decision-making. Stakeholders throughout the value chain make decisions on products and chemicals every day – but are these decisions optimal? With an ever increasing number of criteria to evaluate—price, performance, lifecycle impacts, risk—these decisions pose a significant challenge when considering the many tradeoffs. Recently, alternatives assessment (AA) frameworks have attempted to address these complications, but often do not provide guidance for users to make specific selections. ACC's Sustainability and Market Outreach division is working to solve this issue by incorporating decision science principles into the AA methodology to help users make informed decisions tailored to their preferences. In our demonstration pilot, we use multi-criteria decision analysis (MCDA)—tucked within a user-friendly interface—to guide users as they make decisions in a scientific manner that reflects their preferences and explore tradeoffs between alternatives. ACC populated the online MCDA framework with data from two existing AA assessments: 1) the BizNGO AA for methylene chloride discussed above, and 2) Washington State Antifouling Boat Paint Alternatives Assessment Report, conducted by TechLaw and Northwest Green Chemistry (NGC). ACC also utilized data from other published sources for criteria not considered in these reports. The output is presented using data visualization tools, with the results dependent on user criteria and weightings. To-date, ACC collaborated and received positive and encouraging feedback from academic experts in the field, non-profit organizations, other trade associations, stakeholders within the value chain, and technology providers. We envision that the results of this project will provide a general platform through which stakeholders may perform screening-level assessments and consider tradeoffs for products of interest to them, or may incorporate this framework into their own methodologies and products.

## Pilot Studies – In Search of Innovative Remedial and Restoration Design

### MP076 A Case Study on Adaptive Management and the Optimization of a Pilot Bank Stabilization Remedy for a Mercury-Impacted River

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Historical mercury (Hg) releases occurred at a textile manufacturing facility on the South River, Virginia. These releases resulted in increased Hg concentrations in biotic and abiotic media, which have not declined over the past thirty years, as originally expected. Introduction of legacy Hg impacted soils to the South River through bank erosion is the highest source of Hg loading to the system. In 2009, a bank stabilization pilot (Bank Pilot) was constructed along a 500-ft reach of the South River to test this hypothesis and to assess whether control of Hg loading to the water column and sediment through eroding banks would result in reductions of Hg concentrations in the aquatic environment. Post-implementation monitoring data documented significant mercury concentration reductions in near-bank sediment and pore water samples within three to five years, which demonstrated the effectiveness of the remedial approach. An analysis of remedial alternatives identified bank stabilization techniques similar to those used at the Bank Pilot as the most viable, implementable, cost effective and sustainable remedial option to address Hg loading to the river from eroding riverbanks. Due to the size, linear nature, complexity and spatial variability of the South River system, achieving remedial action objectives identified for the program are best achieved in an adaptive management framework. This approach began with implementation of a series of Interim Measures (IM) at select bank management areas (BMAs) within the first two river miles downstream of the former site. The first IM was completed at the Constitution Park BMA and incorporated learnings from the Bank Pilot project, as well as modifications intended to address landowner preferences, including the preservation of mature riparian habitats. Three IMs have been designed and implemented on the South River to date, with two additional projects currently under design. This presentation will discuss the adaptive management process being used to effectively scale and improve upon an initial pilot study to address mercury loading to the South River from eroding riverbanks.

### MP077 Advances in the Role of Phytotechnologies in Sustainable Site Remediation and Reclamation

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It is becoming increasingly important to develop more sustainable remediation technologies to clean up the literally 1000's of contaminated Brownfield sites within our communities. These are lands that were previously used for industrial or commercial purposes, and have the potential to be reused once they are cleaned up. The traditional remediation strategies for dealing with many types of contaminated soils involve excavation of the soil followed by transport to an off-site treatment facility or to a hazardous waste disposal site. These methods, although expedient, are typically energy-intensive and costly. Furthermore they may affect the environment in other adverse ways through spills during transport, and they disrupt the local ecosystem by removing or destroying the native soil. Increasingly, contaminated site owners, government legislators, and environmental consultants are turning to more 'green' technologies which additionally create new green spaces and decrease $CO_2$ emissions. Phytotechnologies are clearly 'green' technologies, driven by solar energy. In most cases the contaminated soil is remediated in place and is then re-usable. By developing these technologies, we are not simply transferring the liability for contaminated matter from one area (the contaminated site) to another (a landfill). Vegetating the land also acts in our favour in terms of greenhouse gas storage which has been estimated at ~6 tonnes per hectare. Finally, phytotechnolgies can be very cost effective and costs may be spread out over several years. The technology is however very site-specific and it can take years to fully remediate a site using phytotechnologies alone. In many cases, there may be a role they play alongside our more traditional remediation technologies. In every instance, we need to take into account factors including the contaminant type and concentration, the soil make-up, the hydrology of the site, the climate, the native plants at the site, etc. This presentation will highlight examples of phytotechnologies involving metal, organochlorine, petroleum hydrocarbon, and salt-contaminated sites, and will emphasize the integration of plant-based remediation technologies into our portfolio of remediation strategies

### MP078 Concept Maturation of a Coastal Restoration Program Following Successful Implementation of a Pilot Project

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Stratford Point is located at the mouth of the Housatonic River Estuary in Fairfield County, Connecticut. A trap and skeet range was operated at the site from 1926 to 1986 resulting in the discharge of lead shot into surrounding water and sediment. From 2000 to 2011, accumulated lead shot in upland, intertidal, and subtidal areas was removed via excavation. After the excavation, erosion of intertidal sediment exposed residual lead shot at the sediment surface, providing a potential ingestion hazard to foraging waterfowl. In 2014 a 150-foot living shoreline pilot project was constructed as a "proof-of-concept" to demonstrate the potential for living shorelines to decrease erosion of intertidal sediments, reduce potential ecological exposure to contaminants, and restore wildlife habitat. Post-construction monitoring demonstrated that the pilot project effectively promoted sediment accretion, which provided a natural cap over the residual lead shot. Based on these positive results, the pilot project was expanded in November 2016 to protect and enhance an additional 750 linear feet of shoreline. On-going monitoring has demonstrated that, although the living shoreline effectively reduces erosion of intertidal sediments, it is less effective at reducing erosion of the coastal dunes located above mean high water (MHW), which may cause additional lead shot to be revealed. To address this concern, the living shoreline restoration was modified to include additional design elements. In November 2017, oyster cultch (shell) was used to construct a sill, behind which imported sand and soil were placed. Large woody debris was anchored into the sand and soil, which were then planted with *Spartina patens* and *S. alterniflora*. These elements were intended to form a natural, porous medium of shell that would absorb hydraulic energy, promote wave run-up, and generate beneficial vortices, thus reducing wave energy and causing mounds of non-uniform deposition of sand and gravel. Preliminary post-construction monitoring results have been positive. The maturation and evolution of this pilot project concept through construction demonstrates the value of using adaptive management strategies to implement flexible, proactive restoration increasing the likelihood of a successful outcome.

**MP079 Optimizing the sorption of phenol on rice husk powder by immobilised microbial cells**

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Phenols, dyes and heavy metals, are amongst the most important environmental pollutants being faced today. They are typically present in wastewater from industrial processes, such as the petrochemical and pharmaceutical, paper and pulp industries. They contaminate the soil, groundwater, sediments and surface water and their removal from wastewater before discharge into the environment is important. Thus, more efficient and rapid methods of removal and detoxification are urgently needed. This study was aimed at optimizing the efficiency of rice husk as a biosorbent for the removal of phenols and their congeners from liquid wastes and to evaluate their possible application in activated sludge process. In doing so, the elemental and physicochemical compositions of rice husk powder (RHP) to be used as biosorbent were evaluated while microorganisms to be immobilized were isolated from the industrial effluents and thereafter characterized. The selected the microorganism was immobilized on the rice husk powder. The rates of adsorption of phenol on the rice husk powder at different phenol concentrations, biosorbent dose (biomass), pH, temperature, and contact time were determined using Fourier Transform Infrared Spectrophotometer (FTIR). The rate of adsorption of phenol on rice husk powder without immobilized cells was also determined. The adsorption kinetics, equation which best describes the equilibrium uptake and the isotherms which best describes the data obtained were evaluated and the thermodynamic parameters (?G$^o$, ?H$^o$ and ?S$^o$) determined. The elemental analysis of the rice husk powder (RHP) showed the presence of Iron (ppm) 20.396, Aluminum (ppm) 5.478, and Nitrogen 0.923 while the FTIR–spectra of RHP showed the participation of phenols (O-H), amine (N-H), alkyne (C≡C), carbonyl (C=O), aromatic amine (C-N). The biosorption process followed the pseudo first order reaction order with ?G estimated to be -3.772KJ/mol., suggesting that the removal process could be exothermic and spontaneous. More so, the immobilized *Bacillus cereus*on rice husk powder resulted in enhanced removal of phenol and their congeners when compared with the rice husk powder without immobilized cells. This implies that Rice Husk Biomass having immobilized *Bacillus* spp. can be used in activated sludge process for removal of phenols from industrial wastewater.

**MP080 Phytoextraction: A Sustainable Solution to Soils Contaminated with Cement Kiln Dust Leachate**

*A. Litalien, B. Zeeb, Royal Military College / Chemistry and Chemical Engineering Dept*

Cement is a ubiquitous building material with global production approaching 3.3 billion metric tonnes annually. A by-product of cement manufacturing, known as Cement Kiln Dust (CKD) poses significant environmental risk as it is highly alkaline and enriched in salts. Despite this, CKD has been historically disposed of by on-site landfilling and as a result, cement manufacturing plants often have severely salinized soils. Soil salinization decreases both soil quality and vegetative cover, thus negatively impacting local ecosystems. This case study examines the use of plant-based technologies for the remediation of a marshland adjacent to a CKD landfill site at the Lafarge-Holcim plant in Bath, Ontario, Canada. Salt-tolerant plants known as halophytes have been used to stabilize and extract salts from contaminated soil and groundwater, a process known as phytoextraction. Plant species were selected based on their salt tolerance mechanisms: accumulation of salts in above-ground plant tissue, and the secretion of salts on leaf surfaces. While accumulator plants may incorporate large amounts of salts into their tissues throughout a growing season, this biomass requires harvesting and disposal in order to eliminate it from the site. Salts that are excreted however, may be dispersed by wind action allowing for continuous extraction of salts and requiring less maintenance. This study integrates both salt tolerance mechanisms with the aim of optimizing soil chloride extraction and lowering management costs. The ongoing study at the Bath cement manufacturing plant is the first of its kind to implement both accumulator and excretor halophytes for the remediation of salt-contaminated soils. This work will inform future remediation strategies both for the Bath site and for other CKD contaminated regions worldwide. With refined methods, phytoextraction can be a suitable technology for addressing soil salinization.

## Fate and Effects of Metals – Mechanisms of Bioavailability and Toxicity

**MP081 Do DOC characteristics matter? A case study investigating metal toxicity to a tropical freshwater alga**

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Dissolved organic carbon (DOC), common to natural waters, plays a key role in influencing metal speciation and bioavailability. The ability of DOC to alter the toxicity of metals depends on its concentration, source and composition. Each aquatic system is distinct, with a unique combination of organic matter sources, resulting in DOC with complex and heterogeneous chemical compositions. Currently, there is limited research comparing the ameliorative effects of different Australian DOCs on metal toxicity. This information is required if we are to improve bioavailability-based models for derivation and application of water quality criteria for metals. This study involved determining the effect of three distinct Australian DOCs on the toxicity of copper and nickel to a tropical green alga *Chlorella* sp 12. Inhibition of cell division over 72 h, at a range of copper (0-100 µg Cu/L) and nickel (0-2000 µg Ni/L) concentrations was investigated in the presence of each of the DOCs (0, 2 and 10 mg C/L added). Both dissolved and bioavailable metal (using diffusive-gradients in thin-films, DGT) were determined over 72 h. Copper toxicity was reduced in the presence of all DOCs, with up to a 4-fold increase in EC values with increasing DOC concentration, and up to a 2-fold difference in toxicity between the different DOC types. In contrast, DOC concentration or composition had little effect on Ni toxicity to this alga. The amelioration of copper toxicity of the three DOCs varied between wet and dry seasons. Changes in protective ability of the DOCs were significantly related to the aromaticity of the DOCs, with EC values increasing with increasing aromaticity. Potentially bioavailable, or DGT-labile, metal concentrations varied between different DOC treatments. There was good agreement between DGT-labile copper concentrations and the predicted ameliorative effect of the DOC based on its chemical characteristics. DGT-labile copper was highest in the least protective DOC treatment and lowest for the most protective DOC, with up to a 2-fold difference in DGT-labile copper. These results demonstrate that metal bioavailability and toxicity vary with both the source, concentration and composition of DOCs in freshwaters. Thus, the development of protective water quality criteria for metals requires a greater understanding of DOC composition, how composition influences DOC-metal interactions and to what degree these components vary between metals, regions and seasons.

**MP082 The Influence of Major Freshwater Ions on the Toxicity and Kinetics of Silver Nanoparticles in Toxicological Media**

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Silver nanoparticles (AgNPs) are increasing in presence in commercial and medical products due to their bactericidal properties. Aqueous silver ($Ag^+$) toxicity to freshwater organisms has been well studied using the Biotic Ligand Model (BLM). There is strong evidence to suggest $Ag^+$ as the toxic mechanism of action of AgNPs, however toxicity via ionic dissolution from AgNPs is not accurately predicted by the BLM, which suggests the existence of other or additional chemical or biological reactions. Furthermore, consensus on the toxicity of AgNPs has not yet been achieved due the variety of behaviors that AgNPs exhibit depending on their physical properties, such as size or capping agent, or surrounding environmental conditions, such as pH or temperature. As a result, there has been a call to standardize toxicity tests for nanomaterials differently than what is used for other chemicals. The purpose of this research is to observe the effects of the major freshwater ions $Ca^{2+}$, $Na^+$, $Cl^-$, and $SO_4^{2-}$ on the toxicity of AgNPs to *Daphnia magna*. This study uses 8 acute toxicity tests performed in ASTM moderately hard synthetic freshwater that have varying additions of each ion. The BLM predicts that all experimental synthetic freshwaters will reduce $Ag^+$ toxicity in comparison to ASTM moderately hard water without any additions, driven by the concentration of total ions in solution. Nanoparticle kinetic theory also predicts a reduction of dissolved $Ag^+$ concentration caused by a combination of major ion concentration and valency, theoretically reducing $Ag^+$ toxicity from AgNPs. Completed toxicity tests in this study have shown that both of these mechanisms influence toxicity with increases of LC50s much higher than what the BLM predicts for each experimental synthetic freshwater. Factorial analysis is used to determine the specific influence of each freshwater ion on differences in toxicity as well as the measured chemical behaviors: particle concentration and size distribution on ICP-MS, and sedimentation using UV-Vis spectrophotometry. This research aims to examine whether predicted AgNP kinetic behavior occurs in toxicological media and connect how specific particle behaviors influence toxicity in a standardized test. These results could inform nanomaterial-adapted test standards, and also understand the impact of specific major ions in freshwater ecosystems on the environmental toxicity of AgNPs.

**MP083 Toxicity to *Ceriodaphnia dubia* in natural waters with extreme levels of hardness and dissolved organic carbon**

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Most US states use outdated hardness-based criteria to regulate most metals in surface waters. Slow adoption of new criteria based on biotic ligand models (BLMs) reflects uncertainty about whether these models can reliably predict metal toxicity to aquatic biota across wide ranges of water quality conditions affecting bioavailability. BLMs reflect the influence of multiple water quality variables that influence bioavailability, including pH, hardness [calcium and magnesium], and dissolved organic carbon (DOC). BLMs were developed and validated based on tests with wide ranges of water quality conditions, but model validation with natural waters containing extreme values of one or more water quality parameters is still needed. We generated toxicity data (*Ceriodaphnia dubia*) for natural waters representing extremes of water quality in the Midwestern US. We sampled two in each of two study areas: (1) central Illinois, where streams had high hardness (>300 mg/L) and moderately low DOC (2-4 mg/L); and (2) northern Minnesota, where streams had high DOC (>30 g/L) and low hardness (17-27 mg/L). We conducted chronic toxicity tests with waters from two sites in each study area, plus two low-DOC (< 1 mg/L) laboratory waters with hardness adjusted to match each natural water. *C. dubia* tests showed somewhat different trends between the two metals. Nickel EC20s (for reproduction) varied widely (11-fold difference) across the six waters tested, and showed a clear trend for reduced toxicity at higher DOC concentrations. Nickel EC20s were 8.8-12.0 ug/L in the low-DOC lab waters and 66-98 ug/L in the high-DOC Minnesota waters. No protective effect of hardness on nickel EC20s was evident, perhaps because of the prevailing effect of DOC in the soft Minnesota waters. Zinc EC20s showed less overall variation among test waters (5.3-fold) and showed no clear trend for protective effect of either hardness or DOC. Effects of water quality differences on zinc toxicity may have been obscured by the wide confidence intervals for most zinc EC20 values. This uncertainty reflects changes in measured filterable zinc concentrations in exposure chambers during each 24-hour water replacement cycle, which made it difficult to determine which zinc analyses best reflect test organism exposure. Observed differences in toxicity of zinc and nickel will be compared to BLM predictions.

**MP084 Investigation of the Toxicity of Different Metal Species Using an in vitro Approach**

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In solution, metals form different chemical species based on water chemistry and medium composition. It is generally accepted that free metal ions, and, with less efficiency, neutral complexes are the main drivers of toxicity in fish due to their ability to cross the cell membrane. However, recent studies have brought this hypothesis into question. In order to evaluate the bioavailability of neutral and charged metal complexes, as well as free metal ions, exposure media will be designed to allow the formation of a variety of metal species in solution. The rainbow trout (*Oncorhynchus mykiss*) gut cell line (RTgutGC) will be used to investigate the bioavailability of the different metal species. Metal speciation analysis, performed using the chemical equilibrium model Visual Minteq, showed a wide range of chemical species forming among the four metals tested in this study (silver, cadmium, copper and zinc). Following exposure, toxicity, bioaccumulation and bio-reactivity of free ionic, neutral or charged metal species will be measured in RTgutGC. Toxicity will be determined by a multiple endpoint assay measuring simultaneously cell metabolic activity, cell membrane and lysosomal integrity, bioaccumulation by ICP-MS and bio-reactivity by measurement of metallothionein mRNA levels. The metals had distinct affinities to certain anions, for instance: copper showed strong affinity for phosphates and carbonate, and removal of carbonate allowed hydroxyl species to form. Silver and Cadmium showed a high affinity for chloride and zinc remained principally in free ionic state. Preliminary results in cells exposed to Cadmium have shown differing effects among different media. With low chloride media, and phosphorous free media containing carbonate, Cadmium was significantly more toxic in regards to metabolic activity than in the control high chloride synthetic media. Applying both the Visual Minteq results with the toxicity, bioaccumulation and bio-reactivity results will allow a better understanding of which metal species plays the largest role in toxicity, and transport across the membrane. This information could be used to improve bioavailability models such as the biotic ligand model.

**MP085 Toxicity and Bioavailability of Cobalt: Validation of a Cobalt Biotic-Ligand Model**

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It is well known that the bioavailability and toxicity of metals, including cobalt (Co), vary based upon water quality characteristics (e.g., hardness, pH, dissolved organic carbon [DOC]) of freshwaters. In order to account for the changes in the bioavailability of Co to aquatic organisms, extensive toxicity testing has occurred for the development of a Co-specific chronic biotic ligand model (BLM). This model was developed based upon toxicity tests using three aquatic organisms: the fathead minnow, *Pimephales promelas*, the cladoceran, *Ceriodaphnia dubia*, and the green algae, *Pseudokirchneriella subcapitata*, with exposure to

Co concentrations across a range of water quality characteristics (e.g., calcium, magnesium, sodium, pH, DOC). Following development of the Co BLM, a two-phase validation was conducted. A combination of tests in reconstituted laboratory waters and field-collected natural waters were conducted with organisms not used in the BLM development as a model validation exercise. Chronic toxicity tests were conducted using three species: the aquatic plant (*Lemna minor*), a gastropod (*Lymnaea stagnalis*), and a rotifer (*Brachionus calyciflorus*). Two Co-BLM parameter models (one for plants/algae and one for fish/invertebrates) were used to provide model predictions versus observed effects. Observed effects, calculated as 10% effect concentrations ($EC_{10}$s) and 95% confidence intervals around the toxicity values, were considered in evaluating observed versus model predictions. In validation testing of *B. calyciflorus* in laboratory waters, the BLM predicted effects in 8 of 10 tests within a factor of two. In testing with field-collected waters, the model predicted the majority of effect concentrations for *L. minor* (5 of 5 natural waters), *L. stagnalis* (5 of 5 natural waters), and *B. calyciflorus* (4 of 5 natural waters), within a factor of 2. The results demonstrated that the BLM accurately predicts Co toxicity in field-collected natural waters and for organisms not used in the development of the bioavailability model.

**MP086 Role of chloride as a complexing agent in the exposure media on accumulation and bio-reactivity of silver and copper in bluegill fish**

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In general, it is known that fish are less sensitive to metal toxicity in seawater than freshwater due to the higher concentration of salts that make the ionic forms of metals less available to bind with the uptake sites (e.g. the gill and the gut). However, some studies have shown metal toxicity in fish even at high salinity where metals mostly stay in complexed forms. Therefore, it is important to study whether metal complexes can become bioavailable and elicit toxicity. To investigate the accumulation and bio-reactivity of silver and copper complexes in bluegill fish, in this study, we first modelled different exposure solutions varying chloride concentrations (0-200 mM). Visual MINTEQ, a chemical equilibrium model, was used to determine the media composition that allows metal to speciate in different forms (i.e. ionic, neutral complex, and charged complex). Ag speciation was mostly dominated by the ionic form ($Ag^+$) at low chloride concentration (below 0.5 mM). With the increase of chloride, it was dominated by neutral ($AgCl_{(aq)}$) and negatively charged ($AgCl_2^-$) complexes. However, Cu speciation remained constant as $Cu^{2+}$, $Cu(OH)^+$, and $Cu(CO_3)^+$ up to 10 mM chloride concentrations, and after that it was $Cu(CO_3)^+$ that dominated the Cu speciation. Based on these speciation, we selected 0.05, 10, 50, 100, and 200 mM chloride containing solutions for bluegill salinity tolerance experiment. Fish were exposed to different solutions for 96 hours. In the 96-hour salinity exposure experiment, 100% fish mortality were observed at 200 mM solutions (~14.3 PPT). Variable proportions of mortality were found at other concentrations. From this preliminary experiment, we have now chosen three solutions (0.05, 3, and 50 mM chloride) to evaluate accumulation and bio-reactivity (i.e. induction of metallothionein mRNA levels) of different Ag and Cu metal species in bluegill fish (*Lepomis macrochirus*). Fish will be exposed to a sub-toxic concentration of each metal for 96 hours. After exposure, gill, liver, gut, kidney and blood will be collected to measure metal accumulation using ICP-OES and bio-reactivity using qPCR techniques. This study will shed light on the bioavailability of metal complexes in fish.

**MP087 Dynamic variation of metal bioavailability related with responsive mechanisms of molecular biomarkers using in-situ exposure**

*Z. Wang, Jiangxi University of Science and Technology*

Due to the complexity of heterogeneous sediment, the chemical speciation and mobility of metals were in dynamic conditions rather than steady states, resulting in high variability of contaminant bioavailability. Chemical analyses in traditional tests had disadvantages of inaccuracy for contamination characteristics and risk assessments, representing a worst-case scenario in terms of complex exposure dynamics. In this presentation, relevant scientific issues regarding the effects on subcellular distribution and molecular biomarkers with special emphasis on toxic mechanisms and their associations were investigated through powerful experimental designs and field-based manipulations of in situ exposure and kinetic DGT approach. Understanding the degree of pollution and geochemical characteristics of sediment matrix, in situ testing chambers were deployed combining the DGT and caged clam *Ruditapes philippinarum*, and the dynamic exchanges, translocation and mobilization were then clarified among the interface of particles and pore-water in sediments through measuring dynamic parameters and resultant induced fluxes in exposed organism. Integrating a wide battery of biomarkers, dynamic changing processes were assessed, and their interactions were established between exposure and biological effects. Furthermore, variations of transcript expression of functional genes in contaminant-specific biomarkers were obtained through high throughput oligo-DNA Microarray and quantitative reverse transcription polymerase chain reaction in order to elucidate the mechanistic understanding of molecular biomarkers in biological responses. Simultaneously, the approach of in-situ evaluation was established considering the consistency of framework as protocols among sediment chemistry, contaminant bioavailability and adverse effect, which significantly improve accuracy and ecological relevancy in complex exposure situation and thus provide a robust tool to support more comprehensive processes of sediment risk assessment. Overall, the associations not only revealed the fates of accumulated metals, but scientifically favored an improved understanding of exposure and toxic effects in response to subcellular level, supporting the focus of metabolic availability assessment on the intracellular processes or events occurring within organism in future metal biomonitoring. (The author acknowledges financial support by Grant No. 21377125/B070403 from National Nature Science Foundation of China).

**MP088 Transcriptome analysis revealed the effects of different metals levels exerted on *Crassostrea hongkongensis* chronically exposed in wild**

*Y. LI, Hong Kong University of Science and Technology / Division of Life Science; W. Wang, Hong Kong University Sci. Technol. / Department of Ocean Science*

Oysters *Crassostrea hongkongensis* can hyper-accumulate trace metals and survive under high levels of contaminants. We sampled *C. hongkongensis* and identified the transcripts sequences acquired from transcriptome analysis of their gill tissues, coupling with the determinations of metals (Ag, Cd, Cr, Cu, Ni, Pb, Zn). During the long-term chronic exposure, the oysters became blue colors. Our results demonstrated the increase of Zn and Cu transporter expression in oyster soft tissues with the high bioaccumulation of these metals. More than 100,000 genes were assembled *de novo*, and totally 1944 DEGs were identified in the contaminated oysters. Increased expression of genes related to energy production was observed in two contaminated sites, and was significant at the medium contaminated levels. We found severe disruption of cytoskeleton in most contaminated sites. The possible roles of histone modification were also identified in response to metal stress. This study illustrated the molecular mechanisms of metals toxicity in oysters chronically exposed in the filed.

**MP089 Alterations in Some Biochemical Parameters of *Clarias gariepinus* following Subchronic Exposure to Magnesium Hydroxide Nanoparticles**

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The aquatic environment is vulnerable to contamination from engineered nanomaterials. Magnesium nanoparticles (MgOHNPs) have gained commercial interest in the areas of waste remediation and recycling of waste water for portable use. MgOHNPs are among the least investigated nanoparticles and thus their toxicological effects are yet to be unveiled. The nanoparticle was characterized by UV/Vis spectrophotometry, fourier transform infrared spectroscopy (FTIR) and transmission electron microscopy (TEM). *Clarias gariepinus* were exposed to 62.5, 125 and 250 mg/L suspensions of small size (10 nm) MgOHNPs for 21 days under semi-static conditions. Thereafter, they were sacrificed and alterations in the haematological parameters and some biochemical indices in the plasma were examined. The water quality parameters such as chloride ions and dissolved oxygen significantly decreased, while pH, total alkalinity, carbon (IV) oxide, total hardness and nitrate increased significantly in the exposed media compared to the control medium. The results of this investigation revealed a concentration dependent increase in the plasma levels of albumin, total protein, magnesium, calcium, chloride, alkaline phosphatase and acid phosphatase. Whereas, the levels of aspartate aminotransferase, alanine aminotransferase, glucose, total bilirubin, potassium and creatinine decreased significantly. The haematological parameters remained unaltered in the fish at the tested doses. The results indicated that exposure to MgOHNPs could lead to disturbances in blood biochemistry of *Clarias gariepinus*. Key words: Subchronic, Exposure, Nanoparticles, Magnesium hydroxide, *Clarias gariepinus*.

**MP090 Investigating nickel uptake, tissue distribution and depuration in the green shore crab *Carcinus maenas***

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Nickel has been shown to accumulate and exert toxicity to estuarine and marine organisms, but Ni fate and effects in these organisms remain understudied compared to freshwater species. The green shore crab (*Carcinus maenas*) is a widely distributed euryhaline crustacean that is considered a model species for estuarine and marine ecotoxicology studies. Notably, this species has been extensively used to determine impacts of metal contaminants, because of the high capacity of their carapace to retain metals from the ambient water. The main goal of the present study was to provide insights into Ni uptake pathways and depuration strategies in this species. To this end, we characterized the uptake, tissue distribution and depuration of waterborne Ni ingreen crabs exposed for 24 h to 8.2 µg/L of radiolabelled Ni (the USEPA chronic saltwater guideline value). Various exposure conditions were tested to decipher uptake pathways (gills vs. carapace), depuration kinetics, as well as the geochemical vs. metabolic controls on Ni accumulation, including exposures at different temperatures, and carapace shielding experiments. Comparisons with Zn and Ca uptake and depuration dynamics were also made. (IDRC, NSERC Discovery).

**MP091 Uptake and loss kinetics of uranium in an aquatic insect**

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Aquatic insects are the most abundant and diverse macroinvertebrate fauna of freshwater ecosystems. They also play a key role in linking aquatic and terrestrial ecosystems through their metamorphosis and dispersal to riparian habitats from aquatic larvae to winged adults. Adult insects are a vector for the transfer of chemical contaminants to terrestrial consumers, although this varies with the specific chemical. In the case of U, little of the larval body burden is carried by the adult, implying that the risk of U exposure is largely confined to the aquatic ecosystem. However, little is known about the underlying mechanisms governing U bioaccumulation kinetics by insect larvae. We used the precepts of a kinetic bioaccumulation model and experimentally parameterized U uptake and elimination rate constants in a model aquatic insect species, the mayfly *Neocloeon triangulifer*. The model can predict U accumulation and identify potential risk to consumers in impacted ecosystems. Experimental results showed that mayflies efficiently accumulate U from the aqueous phase, but only marginally from diet. Increasing concentrations of U in diet lowered mayfly feeding rates by more than 80%. Nearly 90% of the accumulated U was eliminated within 24 hours. A subsequent U desorption experiment indicated that 60% of the U accumulated after a pulse exposure to waterborne U desorbed within a minute of rinse. In contrast, similar extraction experiments conducted with the stonefly *Zapada* and the Ephemerellid mayfly *Drunella* showed no indication of desorption, suggesting that U was more strongly bound by these two taxa than by *N. triangulifer*. Our results highlight the importance of characterizing species specific processes and avoiding generalization of biokinetic processes to other taxa.

**MP092 Copper Uptake in Periphyton under Natural Stream and Wastewater Effluent Exposures**

*T. Vadas, University of Connecticut / Civil and Environmental Engineering; K. Turpin-Nagel, N. Fuad, University of Connecticut / Department of Civil and Environmental Engineering*

The bioavailability of copper to aquatic organisms is largely dependent on water quality characteristics, which can influence the chemical speciation. In natural waters, a large percentage of copper is found complexed with inorganic and organic ligands leaving a small percentage available as free metal ions. Complexation with dissolved organic matter (DOM) is fundamental in controlling metal speciation and the source of DOM has been shown to influence the binding strength of the ligands. Wastewater treatment plant (WWTP) effluent DOM has been observed to contain high-affinity binding sites for copper and higher percentages of hydrophilic binding ligands leading to enhanced copper complexation. Thus, metal speciation differences are expected to vary between aquatic environments impacted by urban discharges and those more pristine. The aim of the present study was to examine the uptake kinetics of copper to periphyton, a biofilm community of heterotrophic and autotrophic species that grow in running water ecosystems, under freshwater and WWTP effluent exposure conditions. Periphyton were colonized in indoor growth aquariums supplied with freshwater from the Fenton River (Connecticut, U.S.A). Copper uptake kinetic experiments were run using a constructed trickle apparatus, where short-term (90 – 380 minutes) exposures were completed using environmentally relevant total copper concentrations (2 – 16 mg/L) at varying levels of % WWTP effluent. First-order rate kinetics were used to analyze the differences in initial uptake rates between the different exposure waters over the first 30 – 60 minutes of exposure. It was found that as the percentage of WWTP effluent increases in the exposure waters that the periphtyon total and intracellular uptake rates decrease. Reduced periphyton copper content (surface bound and intracellular) was also observed for the exposure waters containing WWTP effluent. Free copper ion concentrations were estimated for the exposure waters by inputting measured water quality parameters and experimentally derived DOM conditional binding constants into Visual MINTEQ. An equilibrium ion exchange technique was used to investigate the exchangeable copper fractions of the exposure waters. Kinetics modeling using the total, free ion, and exchangeable copper concentrations allowed for examination of the bioavailable copper fraction. Measured uptake rates correlated well with those modeled based on exchangeable copper.

**MP093 Effects of Organism Age on *C. dubia* Acute Sensitivity to Zinc in Lab Water**

*S. Vasquez, B.C. Jorgenson, A.M. Briden, N. Lynch, Pacific EcoRisk*

The USEPA acute toxicity testing manual (EPA-821-R-02-012) requires that *Ceriodaphnia dubia* be less than 24 hours old at test initiation. Water effect ratio (WER) testing in accordance with the USEPA "Interim Guidance on Determination and Use of Water-Effect Ratios for Metals" (EPA-823-B-94-001) is performed following the test conditions outlined in EPA-821-R-02-012, including the requirement of < 24-hour old *C. dubia*. An assessment of zinc $EC_{50}$ values for 48-hour acute *C. dubia* lab water tests performed by Pacific EcoRisk (PER) showed substantial variability in lab control water $EC_{50}$ effect thresholds, with some values outside a factor of 1.5 of the mean of the PER database (hardness-normalized to 50 mg/L). Following this observation, a study was designed to see if this variability could be reduced in order to achieve more consistent and predictable $EC_{50}$ values for zinc in lab water. Organism age was investigated as a source of this variability; to investigate this potential source, tests were initiated with organisms of blocked age. Younger aged organisms showed improved test performance, with lower overall intratest and intertest variability. Results of zinc in lab control water are also compared with copper.

**MP094 Fish otoliths as tracers and records of inorganic contamination exposure – testing the key assumption**

*J. Brandt, USGS / Nicholas School of the Environment; M.R. Ross, Colorado State University; E.S. Bernhardt, Duke University / Biology; R.T. Di Giulio, Duke University / Nicholas School of the Environment*

Otoliths are acellular, carbonate structures located in the inner-ear cavity of teleost fishes. While their primary functions include balance, motion sensing, and hearing, their metabolic stability and approximately daily accretion rate also allow them to “record” certain aspects of a fish’s life-history. Historically, this characteristic has been exploited by population biologists and those studying the movement of migratory fish in and out of freshwater environments. More recently, ecotoxicologists have also incorporated otolith analyses into their assessments of polluted sites. Much of the work in this area has rested on the key assumption that inorganic water chemistry dictates that of otoliths and we tested this assumption with two independent projects. In the first, we showed that the $^{87}Sr/^{86}Sr$ isotope ratio of surface waters from a reservoir receiving coal combustion residual (CCR) effluents (~0.71013) was similar to that determined for largemouth bass otoliths collected from the same reservoir (~0.70996) and both ratios were statistically higher than those in corresponding samples from a nearby reference lake (all $p < 0.0001$). In the second, we compare time series otolith concentrations of five CCRs to overlapping time series of CCR loading from the associated coal-fired power plant. Here, we tested whether dramatic changes in water chemistry (e.g., a decrease in selenium (Se) loading from max rate of 2664 g/day to 124 g/day) were reflected by otolith chemistry. We observed substantial time lags between the two phenomena, differences within a given species, and differences among three species with distinct ecomorphologies, all of which challenge the assumption that inorganic water chemistry determines otolith chemistry for all elements. Together, our results indicate that (a) the shared Sr chemistry between water and otoliths from a CCR-receiving reservoir introduces otoliths as novel bio-tracers of contaminant sources, but that (b) otolith chemistry of some elements, like Se, additionally reflects dietary exposure. Ideas for an upcoming project to determine the relative contributions of water and dietary exposures to otolith chemistry—with the ultimate goal of developing a predictive model—will also be discussed. Drawing on fish physiology, exposure geochemistry, and hydrology, this work considers the biological fate of metals with potential applications and implications for aquatic contaminant monitoring going forward.

**MP095 Tissue Metal Concentrations in Atlantic Sharpnose Shark**

*R. Somerville, Jacksonville University / Marine Science; J. Gelsleichter, S. Ehnert, University of North Florida / Biology; G.K. Bielmyer-Fraser, Jacksonville University / Chemistry*

Metals occur naturally in the environment; however, anthropogenic practices have resulted in increased metal concentrations in sediments, waterways, and biota. Sharks are important species, both recreationally and commercially. Sharks occupy a high trophic level; and, tissue metal concentrations in these organisms may reflect metal transfer in aquatic food webs. Extensive research has been conducted on mercury toxicity in sharks; however, reference levels of other metals have not been established in many species. In this study, concentrations of nickel, copper, lead, cadmium, selenium, silver, zinc, and aluminum were measured in the muscle tissue of *Rhizoprionodon terraenovae* (Atlantic sharpnose shark),collected from Virginia to Texas. A total of 165 samples were analyzed for correlations among metals, size/age, gender, and location of capture. With the exception of mercury, there are no published data on tissue metal concentrations within this species along the eastern United States. This study provides reference levels of metal contaminants in the muscle tissue of Atlantic sharpnose sharks and provides insight into metal accumulation in this higher-level carnivore.

**MP096 Fate and distribution of cobalt in freshwater microcosms**

*M.C. Cavallaro, Oregon State University / Department of Environmental and Molecular Toxicology; E. Stefansson, Oregon State University; A.S. Cardwell, B.A. Stubblefield, Oregon State University / Environmental and Molecular Toxicology*

A series of aquatic microcosms were used to evaluate the fate and distribution of dissolved cobalt (Co) within a freshwater community. Nineteen 100-L microcosms were established with field-collected lake sediment and inoculated with laboratory and wild populations of algae, macrophytes, plankton, and benthic invertebrates. The study consisted of five nominal cobalt treatments: 0.5, 2.5, 12.6, 62.9, and 315.5 µg Co/L ($n$ = three microcosms per treatment) with four control microcosms. Water quality parameters and cobalt concentrations in water, pore water, sediment, and biota were measured throughout a 56-day exposure period. Rapid losses of cobalt from the water column were observed during the first 14 days of the study, and microcosms were re-spiked daily to maintain the target nominal concentrations. During this state of flux, rapid loss of Co in the water column was analytically confirmed as a result of biological uptake, presuming a state of equilibrium was ultimately reached. Water column Co concentrations were strongly correlated to biota Co tissue concentrations (correlation coefficient; $r = 0.88$), more so than sediment ($r = 0.44$) or pore water ($r = 0.38$) Co concentrations. High bioaccumulation of cobalt was found, particularly in the primary producers. At the termination of the test, mean measured (± SD) cobalt concentrations in highest treatment for *Lemna minor*, *Elodea canadensis, Myriophyllum pinnatum, a*nd mixed periphyton taxa were 4860 (± 1593), 4401 (± 459), 890 (± 25), and 1904 (± 309) µg/g dw Co, respectively. These results suggest that under these test conditions (i.e., closed, static system), primary producers display an enhanced ability to accumulate Co. Invertebrate community data indicate effects to cladoceran populations at exposures > 62.9 µg Co/L, with concomitant increase in phytoplankton likely due to decreased grazing pressure. Data generated from the present study improved our understanding of the fate and distribution of dissolved cobalt and highlight the potential indirect impacts chronic cobalt exposure may have on primary consumers.

**MP097 Use of benthic community structure and metal concentrations in biota to assess the effectiveness of mine reclamation activities**

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Concentrations of metals in streams are typically assessed through the collection of surface water grab samples over short durations. This sampling approach may not reflect actual conditions, especially when inputs of metals are episodic or seasonal. Measuring changes in community structure associated with metals concentrations in benthic biota may represent a better way to assess management actions that prevent metal contaminated water from entering streams. In 2013, the Washington State Department of Ecology implemented a study in a metal contaminated stream impacted by historic copper mining activities, using the above approach to assess the effectiveness of mine remediation efforts. Initial results indicate that metals concentrations in sediment and surface water did not correlate well with remedial actions, concentrations of metals in periphyton, or changes in a measure of macroinvertebrate health. However, metals concentrations in periphyton did responded predictably to remedial actions meant to eliminate surface and subsurface discharges of metal contaminated water to the creek. Additionally, macroinvertebrate health, as indicated by an index of biological integrity, tracked well with metals concentrations in periphyton collected at the same locations. These results suggest biological communities have great potential for tracking remediation efforts at sites impacted by elevated inputs of metals.

**MP098 Trace Element Concentrations in Cetaceans Taken for Human Consumption off St. Vincent, West Indies**

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Cetaceans are long-lived top predators which can accumulate high concentrations of trace elements in their tissues. In St Vincent, several small cetacean species are taken for human consumption each year. This study investigated the concentration of As, Cd, Cr, Hg, Pb, and Se in 4 tissues (muscle, blubber, kidney, liver) from 5 species of cetaceans [false killer whale (*Pseudorca crassidens*), killer whale (*Orcinus orca*), Risso's dolphin (*Grampus griseus*), short-finned pilot whale (*Globicephala macrorhynchus*, and unidentified dolphins from the Stenella genus (*Stenella* spp.)] using microwave acid digestion and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analysis. There was a large intra- and interspecies variation in trace element concentrations. The highest mean concentration of Pb (1.22 µg/g dry wt), As (8.66 µg/g dry wt) and Cr (4.21 µg/g dry wt) were found in *G. macrorhynchus* liver, *P. crassidens* liver and *O. orca* muscle, respectively. Hg, Cd, and Se were found at highest concentration in the liver, followed by kidney and muscle, and lowest in the blubber in *P. crassidens*, *G. macrorhynchus* and *O. orca*. The mean liver Hg concentration in *O. orca* and *P. crassidens* was > 1,000 µg/g dry wt, and some individuals had a concentration that exceeded 3,000 µg/g dry wt. For all species, there was a strong correlation ($R^2 > 0.96$) between Hg and Se concentrations in the muscle, kidney, and liver. All muscle samples had a Se:Hg molar ratio < 1, whereas all blubber samples had a Se:Hg molar ratio > 1; this indicates that Se may have a protective role against Hg toxicity for blubber, but not for muscle. With the exception of *G. griseus*, all species had an average muscle, blubber, liver and kidney Hg concentration, and average muscle, liver, and kidney Cd concentration that exceeded the *Codex Alimentarius* Commission Food Standards Program (FAO and WHO) Hg and Cd recommendation of 1.0 µg/g wet wt and 0.1µg/g wet wt, respectively. Local government officials in St. Vincent should consider the concentration of trace elements, along with cultural practices and economic impacts when making recommendations regarding the consumption of cetaceans.

## Indoor Human Exposure to Particulate Matter and Persistent Toxic Chemicals: Emission Patterns, Fate and Health Risk

**MP099 Kinetics of Brominated Flame Retardant (BFR) Releases from Granules of Waste Plastics**

*B. Sun, Jinan University*

Plastic components of e-waste contain high levels of brominated flame retardants (BFRs), whose releases cause environmental and human health concerns. This study characterized the release kinetics of polybrominated diphenyl ethers (PBDEs) from millimeter-sized granules processed from the plastic exteriors of two scrap computer displays at environmentally relevant temperatures. The release rate of a substitute of PBDEs, 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), from the waste plastics, was reported for the first time. Deca-BDE was the most abundant PBDE congeners in both materials (87−89%), while BTBPE was also present at relatively high contents. The release kinetics of BFRs could be modeled as one-dimensional diffusion, while the temperature dependence of diffusion coefficients was well described by the Arrhenius equation. The diffusion coefficients of BFRs (at 30 °C) in the plastic matrices were estimated to be in the range of $10^{-27.16}$ to $10^{-19.96}$ $m^2 \cdot s^{-1}$, with apparent activation energies between 88.4 and 154.2 $kJ \cdot mol^{-1}$. The half-lives of BFR releases (i.e., 50% depletion) from the plastic granules ranged from thousands to tens of billions of years at ambient temperatures. These findings suggest that BFRs are released very slowly from the matrices of waste plastics through molecular diffusion, while their emissions can be significantly enhanced with wear-and-tear and pulverization.

**MP100 Organic Flame Retardants in Egyptian Indoor and Outdoor Environments: Levels, Sources and Implications for Human Exposure**

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Little is known about the presence of the polybrominated diphenyl ethers (PBDEs), alternative flame-retardants (NFRs) and organophosphate flame retardants (OPFRs) in developing countries. This study investigated – for the first time – concentrations, sources and exposure levels of PBDEs, NFRs and OPFRs in the indoor and outdoor environments of Alexandria, Egypt, in dust and gas-phase samples. Passive samplers were deployed (n = 78) to determine gaseous concentrations, and various dust samples were collected from apartments (n = 25), working places (n = 14), cars (n = 18), and outdoors (OD, n = 30). Indoor gaseous (PBDEs: 7.0 – 300 pg/$m^3$; NFRs: 0.20 – 13 pg/$m^3$; OPFRs: 7.0 – 64 pg/$m^3$) and dust concentrations (4.0 – 770 ng/g; 0.50 – 8.5 ng/g; 150 – 1,850 ng/g) were significantly higher than outdoor (0.20 – 41 pg/$m^3$ and 0.50 – 475 ng/g) concentrations. BDE-47, 99, HBB, BTBPE and DDC-CO, tris-1,3-dichloropropyl phosphate (TDCPP), tris(1-chloro-2-propyl) phosphate (TCPP), tri (2-butoxyethyl) phosphate (TBEP) and triphenyl phosphate (TPP) dominated in all samples with more indoor variabilities. Profiles of PBDEs, NFRs and OPFRs in OD and floor dust were similar but differed from elevated fine dust, possibly due to the influence of carryover of OD by shoes. Although factors such as number of electronics, number of foamed furniture, construction year and floor type significantly correlated with the majority of PBDE congeners, chlorinated OPFRs and some NFRs in apartments and working places, sources were not clearly identified for NFRs. Significant log-linear relationships were observed between dust-air partitioning coefficients and octanol – air-partitioning coefficients in all microenvironments, indicating an equilibrium partitioning between dust and vapor. Exposure assessment indicated low possibility of occurrence of adverse health effects, with the inhalation pathway (for adults) and dust ingestion (for children) acting as the more important exposure routes.

**MP101 Phthalates, PAHs and Hopanes in Dust Collected in Homes Involved in the Canadian Healthy Infant Longitudinal Development Study**

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Exposure to semi-volatile organic compounds (SVOCs) including phthalates and polycyclic aromatic hydrocarbons (PAHs) may be associated with the development of childhood asthma. Phthalates have a wide range of consumer applications including plasticizers, fragrance "keepers", adhesives and sealants, paints and coatings, plastic and rubber materials and other products largely found in the indoor environment. PAHs are produced from incomplete combustion and have some petrogenic sources with dominantly indoor sources including cigarette smoke, emissions during cooking, and fireplaces. The Canadian Healthy Infant Longitudinal Development (CHILD) Study is a population-based birth cohort examining the origins of asthma and allergic disease. We present results characterizing phthalates, PAH and hopanes in dust collected from CHILD Study homes when the infants were 3-4 months of age. The concentration of phthalates and PAHs in dust is hypothesized to be a useful indicator of exposure for epidemiological analyses examining possible associations with the development of asthma and other related outcomes. Pregnant mothers were enrolled in the CHILD study between 2009-2012 from 4 cities and 1 rural area in Canada. Dust samples were collected from the floor of the most used room and bedroom by trained research assistants using a standardized protocol. Children underwent clinical assessments and were phenotyped at 1, 3 and 5 years. Archived dust samples were analyzed for 6 phthalates, 16 PAHs and 12 hopanes. Hopanes are present in engine lubricants and were included to estimate exposures related to traffic air pollution in the home. Preliminary results show that levels of these compounds differ among homes; in general, the concentrations of phthalates were greater than levels of PAHs which in turn were greater than levels of hopanes. House dust concentrations of SVOCs may be suitable proxies for childhood exposures for epidemiological analyses of this cohort.

## Chemistry and Exposure Assessment – Posters

**MP102 Energy Dispersive X-ray Spectroscopy Mapping of Particles As A Component of Lichen Biomonitoring in Seattle, Washington**

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The composition of airborne particulate matter (PM) can be used to determine the identity and contribution of overlapping air pollution emissions. One limitation of this technique is the cost of filtering PM at enough locations to give meaningful spatial data. We address this limitation by using *Ramalina farinacea* to collect PM deposition across 9 locations in South Seattle. This was used in tandem with metal bioaccumulation to identify driving factors of air quality in each of the sample sites. Bioaccumulation was measured using sequential extraction of lichen tissue with inductively coupled plasma-mass spectrometry for 24 metals. Particles were characterized using backscatter scanning electron microscopy with energy-dispersive X-ray spectroscopy mapping to determine the size and composition of 17,783 particles. The metal and PM accumulation data were analyzed using positive matrix factorization for source apportionment, which was interpreted with the SPECIATE repository to identify major emission contributions. Our findings demonstrate that lichen can successfully be used to capture PM for electron microscopy analysis, and have identified vehicular emissions as the primary driver of PM trends in South Seattle.

**MP103 Uncertainty estimation of DTT measurement of Oxidative Potential of Ambient Aerosols**

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Particle matter (PM) has been linked to adversed health effects even at low concentrations. The most accepted hypothesis describes PM-induced inflammatory processes derived from oxidative stress as a result of the generation of an excess of reactive oxygen species (ROS) and radical species that exceed the availableantioxidant defenses producing cell damage. Thus, oxidative stress represents a relevant mechanism of toxicity derived from PM, and the measurements of the oxidative potential (OP) in PM could be a first step in the elucidation of the subsequent downstream processes. Different assays exist for measuring OP, the most common being the dithiothreitol (DTT) assay, which is currently used in studies for the measurement of oxidative potential (OP) of PM. The present work presents a measurement uncertainty evaluation according to Guide to the Expression of Uncertainty in Measurement (GUM) of the OP by DTT assayin PM10, as measured by UV-VIS Method. The GUM method is not typically used to report uncertainty. In general, the analytical results only report the measurement's standard deviation under repetition as an uncertainty; thus, not all sources of uncertainty are considered. In this work, the major sources of uncertainty regarding the measurements were identified. Acknowledgments.One of the authors (MALG) acknowledges the support of National Commission for Scientific and Technological Research CONICYT/FONDECYT 2016 grant no. 1160617. The authors declare no financial relationships with any organizations that might have an interest in the submitted work; no other relationships or activities that could appear to have influenced the submitted work.

**MP104 Toxic effects of the emissions from burning tyres on abattoir workers in Nigeria**

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In the bid to reduce the littering of our environment of condemned tyres, they are now highly used as a regular practice for roasting/processing of slaughtered animals such as goats, sheep and cows, especially their edible skin popularly known as "kanda" in the local parlance. Burning tyres release particulate matter, volatile organic compounds (VOCs) and hazardous air pollutants (HAPs) especially polycyclic aromatic hydrocarbons (PAHs) into the environment. PAHs are reported to cause chest pain, respiratory irritation, cough, dermatitis, depressed immune system, a decrease in lung function, and also carcinogenic, following chronic exposures. This study was aimed at evaluating the risk of exposure of abattoir workers (butchers) to emissions from burning tyres during roasting/defurring of cows and goats within the abattoir premises. Specifically the study sought to determine the: (i) concentrations of some selected EPA priority PAHs in ambient air in, and around the abattoir, (ii) concentrations of particulate matter in ambient air in and around the abattoir, (iii) concentrations of some PAH metabolites and some heavy metals in post-shift urine of butchers and control subjects, (iv) spirometric evaluation of lung function, (v) concentration of urinary phenol and (vi) cytogenetic evaluation of DNA damage in buccal exfoliates of butchers. Some of the results show that the concentration of 1-Hydroxypyrene (1-OHPyr) (µg/molCret), a PAH metabolite, in the post-shift urine samples of the butchers was significantly higher, ($P < 0.05$) with mean concentration ±SD value of (0.52±0.13µg/molCret vs 0.20±0.07µg/molCret); urinary phenol concentration, (14.26 ± 1.19 mg/L vs. 4.44 ± 1.12 mg/L); and concentrations of the heavy metals zinc and nickel (0.91±0.19 vs 0.31±0.28 mg/l and 0.11±0.06 vs 0.06±0.02 mg/l, respectively) in exposed butchers when compared with the control which were unexposed administrative staff working in the abattoir respectively. The buccal epithelial exfoliates showed that karyorrhexis and condensed chromatin bodies were significantly higher in the exposed butchers than in the unexposed control.

**MP105 Study on the pollution characteristics of polychlorinated biphenyls in PM2.5 of different cities in China**

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This study is to evaluate the distribution and concentration of polychlorinated biphenyls in $PM_{2.5}$ , and to monitor the temporal trend, to identify the source of PCBs for providing information to control and manage PCB pollution problem. The samples were collected with filters using high volume sampling in four cities including Shanghai, Beijing, Guangzhou and Harbin from November 2015 to March 2017. The 18 polychlorinated biphenyls (PCBs including 12 coplanar PCB77, 81, 126, 169, 105, 114, 118, 123, 156, 157, 167, 189; and 6 indicator PCB28, 52, 101, 153, 138, 180) were analyzed with high resolution gas chromatography-high resolution mass spectrometry (HRGC-HRMS) based on USEPA 1668C method. The result showed that the mass concentration of PCBs in $PM_{2.5}$ were in the range of 24.0-40.5 pg/m$^3$, the toxic equivalent quantities (TEQs) of PCBs in $PM_{2.5}$ were in the range of 0.105-0.680 fg WHO -TEQ/m$^3$. Compared with studies from large cities in other countries, the concentration of

PCBs is close to the level in Manchester, lower than Chicago and Paris and slightly higher than Tokyo. The distribution and temporal trend of Polychlorinated biphenyls in $PM_{2.5}$ in four cities in China are quite different, and indicator PCBs were the most abundant contributor to the total concentration of PCBs in all cities. Compared the congener profile of the PCBs in $PM_{2.5}$ and the ones from automobile exhaust, emission of combustion of fossil fuel and emission from industry sites, we concluded that automobile exhaust is the main source of PCBs in Shanghai, with relative high level of PCB 28 and 52; The main source of PCBs in $PM_{2.5}$ from Beijing is from the incomplete combustion of fossil fuel consumed in the industry. No clear dominant PCB congener were found from industry sites and the highest PCBs concentration was observed in industrial sites followed by the reference urban sites and residential area. Relative high PCB 118 and 153 in $PM_{2.5}$ from GuangZhou matched the emission profile from waste incineration and burning plastics with chlorine. Concentration of PCBs in $PM_{2.5}$ in Harbinis lower than other three cities. The PCBs level increased in GuangZhou, while decreased in ShangHai and Beijing and no change in Harbin during the monitoring period.

**MP106 Calculation of the sampling rate of PCB congeners using the passive air sampling method (PASM)**

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The sampling rate ($m^3$/day) of the passive air sampling method (PASM) was calculated for measuring the concentrations of PCBs and dioxins (DXNs) in ambient air. Generally, we utilize the active air sampling method (AASM) for monitoring the atmospheric concentrations of PCBs and DXNs. The AASM requires an expensive air sampler and requires electricity, while the PASM is cost-effective and requires no energy. Therefore, the PASM is better suited to monitoring organic pollutants in a wide area than the AASM. We set a flying-saucer-shaped passive air sampler on the rooftop of our laboratory in Sapporo city, Japan. An AASM sampler was set next to the PASM sampler. A quartz fiber filter (QFF), two polyurethane foam plugs (PUF), and an activated-carbon felt disk (ACF) were settled in the AASM sampler, whereas only one PUF was settled in the PASM sampler. The sampling period for the PASM was two months. The PCB concentration was analyzed via high-resolution gas chromatography (HRGC)/high-resolution mass spectrometry (HRMS). Considering the five PASM samples, from April 2017 to January 2018, the total PCB concentrations ranged from 14,000 to 58,000 pg/PUF. The sampling rates of the major PCB congeners by the PASM were calculated in comparison to those of PASM and AASM. The average sampling rates of six PCB congeners were 2.1 $m^3$/day (PCB-11), 3.1 $m^3$/day (PCB-28), 3.7 $m^3$day (PCB-52), 4.5 $m^3$/day (PCB-101), 5.1 $m^3$/day (PCB-153), and 4.8 $m^3$/day (PCB-179). These results were similar to those reported in other countries. Therefore, PASM is suitable for monitoring the concentration of organic pollutants in ambient air in various regions.

**MP107 Characteristics of contamination for HCH and endosulfan in air and soil in the Republic of Korea**

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Introduction HCH and endosulfan is an organochlorine pesticides (OCPs) that has widespread use in many parts of the world, including the European Union, Australia, Canada, United States and Republic of Korea. OCPs has been used from 1940s and is effective against a broad number of insect pests and mites. As a result, this pesticide is applied to a wide number of crop types including cotton, fruit trees and plantation crops such as tea and coffee. However, due to its semi-volatility and relative persistence, OCPs is a ubiquitous environmental contaminant occurring in many environmental compartments. This study was performed to understand the actual isomer ratio of HCH and endosulfan in ambient air and soil of Republic of Korea. Materials and methods The air and soil samples were selected at 108 sites. Air samples were collected using high volume air sampler for 24 hours per day for 3 consecutive days with 0.75 m3/min flow rate. Particles on glass fiber filter and gaseous phases in activated carbon felt/polyurethane form were extracted by soxhlet extraction with dichloromethane, toluene and acetone for 24 hours. Soil samples were mixed and collected 100 g as dry weight, and a extracted by soxhlet using toluene for 24 hours. OCPs were cleaned up by florisil cartridge. 1st fraction of cartridge was activated with 10mL of n-hexane and then eluted with 100mL of n-hexane, 2nd fraction with 100mL of n-hexane solution containing dichloromethane (25% vol) was received. HCH and endosulfan were analyzed by HRGC/HRMS. Results and discussion From the results of HCH and endosulfan analysis in air and soil, β-HCH is predominant isomer in the soil. In HCH, β-HCH is the most stable and persistent in soil due to lower vapour pressure than other HCH isomers, which make for β-HCH easier adsorption to the soil and less evaporative loss from the soil. And endosulfan-sulfate is predominant isomer in the soil. These findings are in concordance with the endosulfan metabolic rate. Endosulfan is microbially transformed to the stable and toxic metabolites β-endosulfan and endosulfan-sulfate in the soil due to several factors such as soil type, temperature, moisture and organic carbon content. Also, this study suggests that HCH and endosulfan of contamination were affected by seasonal temperature variations. Because Republic of Korea has four distinct seasons, and the temperature difference between winter and summer is about 40 degress on average.

**MP108 Volatilization and Atmospheric Transport of Chlorpyrifos and Chlorpyrifos Oxon under Field Conditions**

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Vapor drift occurs when pesticides volatilize from sprayed fields and are blown off-site by the wind, potentially affecting non-target ecosystems. Organophosphate insecticides can travel many kilometers through the atmosphere and due to their toxic and moderately persistent nature, they may incur damage away from the application site. The objective of this work was to better predict the transport and fate of the organophosphate insecticide, chlorpyrifos and its degradation product, chlorpyrifos oxon. To this end, we sprayed a commercial formulation of chlorpyrifos on a field of Purple Tansy (*Phacelia tanacetifolia*) at a farm near Ida Valley, Central Otago, New Zealand in January 2017. Air samples were collected using one high-volume air sampler (HVAS) and seven low-volume, battery-powered active air samplers (LVASs). Air samplers were run for 6 hours per sample and were deployed in a grid around the field and along a transect extending up to 500 m away from the sprayed field. Sampling took place for 21 days after spraying. Soil samples were also collected from inside and outside the sprayed field throughout the study period. We quantified chlorpyrifos and its oxon in the air and soil samples and used temperature, relative humidity, wind direction and precipitation to understand the concentration trends with time and space. Chlorpyrifos was detected every day of the sampling period, with concentrations decreasing significantly following three days after spraying. Chlorpyrifos oxon was only detected in samples from the HVAS within the first three days of spraying. Precipitation and rainfall played an important role in controlling the measured concentrations. Chlorpyrifos volatilization rates from the field were calculated with a pesticide volatilization model and the characteristic travel distance (CTD) of chlorpyrifos was calculated using the ELPOS (Environmental Long-range Transport and Persistence of Organic Substances) model, enabling generation of modeled pesticide concentrations over space and time. Measured chlorpyrifos concentrations were also compared to those generated with the SCREEN3 model, a screening version of the Industrial Source Complex Dispersion Models (ISC3). Results from this work are useful in predicting the distances and time after spraying at which chlorpyrifos drift can cause potential harm to non-target organisms.

**MP109 Impact of Simulated California Rice-Growing Conditions on Chlorantraniliprole Partioning**

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Chlorantraniliprole (3-Bromo-N-[4-chloro-2-methyl-6-(methylcarbamoyl) phenyl]-1-(3-chloro-2-pyridine-2-yl)-1H-pyrazole-5-carboxamide; CAP; water solubility 1.023 mg?$L^{-1}$) was recently registered for application on California rice fields. Air- and soil-water partitioning of CAP were investigated under simulated California rice field conditions through calculation of $K_H$ and ?$_{aw}$H and a batch equilibrium method following OECD 106 guidelines, respectively. $K_H$ and ?$_{aw}$H were determined to be 1.69x$10^{-16}$ – 2.81x$10^{-15}$ atm?$m^3$?$mol^{-1}$ (from 15 – 35 °C) and 103.68 kJ?$mol^{-1}$ respectively. Log($K_{oc}$) ranged from 2.59 – 2.96 across all soil and temperature treatments. Log($K_F$) ranged from 0.61 – 1.14 across all soil, temperature and salinity treatments. Temperature and salinity increased sorption significantly at 35 °C ($P < 0.05$) and 0.2 M ($P < 0.0001$) respectively, while soil properties impacted sorption across all treatments. Overall results, corroborated using the Pesticides in Flooded Applications Model, indicate that volatilization of CAP is not a major route of dissipation and sorption of CAP to California rice field soils is moderately weak and reversible. DOI:10.1021/acs.jafc.7b05775

**MP110 Photochemical Degradation of Polyhalogenated Carbazoles in Hexane by Sunlight**

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Polyhalogenated carbazoles (PHCZs) are a class of halogenated dibenzopyrrole. In recent years, they have been increasingly detected in the environment and found to be bioaccumulative and potentially toxic. However, their environmental transformation potential is largely unknown. In this study, the UV absorption spectra with wavelength range 200-500 nm were obtained for unsubstituted carbzaole (CZ) and 10 individual chlorinated (3-CCZ, 1368-CCZ, 2367-CCZ), brominated (3-BCZ, 27-BCZ, 36-BCZ, 136-BCZ, 1368-BCZ), and mixed halogenated (1-B-36CCZ, and 18-B-36-CCZ) PHCZ congeners in hexane. In addition, individual hexane solutions were prepared in clear glass vials for CZ and these PHCZs as well as a sediment extract involving 9 other PHCZs for which the molecular formulas were determined but the halogen substitution positions remain unknown. The solutions were exposed to natural sunlight along with corresponding controls in amber glass vials, to investigate the kinetics and pathways of PHCZ photodegradation. All targeted compounds decomposed rapidly under solar irradiation, which indicates that photolysis is a possible route of environmental loss of PHCZs. The pseudo-first-order reaction rate constants ($k$) of these PHCZs varied from 0.183 $h^{-1}$ to 2.394 $h^{-1}$, and increased exponentially with an increasing number of chlorines and bromines in PHCZ molecules. A predictive equation was obtained by multi-variant linear regression of ln $k$ with the numbers of bromine and chlorine as independent variables, which showed that contribution to ln $k$ from each bromine atom is more than doubling of that from each chlorine atom. Iodine-containing PHCZs decompose more rapidly than those without iodine, suggesting the loss of iodine is rapid and likely to be the first step. The occurrence of stepwise debromination was confirmed by examining the reaction products for six PHCZs containing bromines. To quantitatively determine the contribution of dehalogenation to the total photolytic degradation, we adopted a kinetic model for simplified parallel reactions. The modeling results indicated that debromination contributed only 20% to 51% of the total loss of the parent PHCZs. Further research is needed to identify and quantify the contributions of other photolytic degradation routes of PHCZs in hexane.

**MP111 Adaption and use of an inexpensive quadcopter of targeted sampling of mercury and VOCs in the atmosphere**

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We modified a popular and inexpensive quadcopter to collect gaseous mercury (Hg) on gold-coated quartz cartridges, and analyzed the traps using cold vapor atomic fluorescence spectrometry. For VOCs we conducted active sampling on sorbent tubes outfitted to the quadcopter and analyszed them by thermal desorption (TD) gas chromatography-mass spectrometry (GC-MS). The aerial sampling method permits for the simultaneous collection of multiple air samples on separate tubes concurrently, increasing sample throughput compared to single canister sampling. Furthermore, the method is relatively inexpensive when compared to similar approaches, with overall costs below about $2000 (U.S. dollars). Flight times averaged 16 minutes, limited by battery life, and yielded >5 pg of Hg, well above the limit of detection (< 0.2 pg). We measured progressively higher concentrations upon both vertical and lateral approaches to a dish containing elemental Hg, demonstrating that the method can detect Hg emissions from a point source. Using the quadcopter, we measured atmospheric Hg near anthropogenic emission sources in the mid-south USA, including a municipal landfill, coal-fired power plant (CFPP), and a petroleum refinery. Average concentrations (± standard deviation) immediately downwind of the landfill were higher at ground level and 30 m compared to 60 m and 120 m (5.3 ± 0.5 ng $m^{-3}$, 5.4 ± 0.7 ng $m^{-3}$,4.2 ± 0.7 ng $m^{-3}$, and 2.5 ± 0.3 ng $m^{-3}$, respectively). Concentrations were also higher at an urban/industrial area (Memphis) (3.3 ± 0.9 ng $m^{-3}$) compared with a rural/background area (1.5 ± 0.2 ng $m^{-3}$). For VOCs, we measured at several heights near anthropogenic sources in the mid-south USA, including a municipal landfill, petroleum refinery, and a coal fired power plant (CFPP), and within the canopy of a loblolly pine (*Pinus taeda*) forest. Concentrations of benzene, toluene and xylene (BTX) were higher ($p< 0.05$) downwind of the refinery and CFPP compared to upwind. We observed both a unique mixture of VOCs at each site and higher concentrations of abundant VOCs downwind compared to upwind of the point sources, and within versus above the forest canopy. Overall, this feasibility study demonstrates that highly maneuverable multicopters can be used to probe Hg and VOC concentrations aloft, and thus have great potential to be utilized in unique sampling situations and for vertical profiling.

**MP112 Testing the Use of Wastewater Tracers as Predictors of Pharmaceutical Distributions in The Long Island Sound Estuary**

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Urban estuaries such as Long Island Sound (LIS) receive large volumes of treated effluents from numerous municipal wastewater treatment facilities. These effluents contain many classes of contaminants including pharmaceutical compounds. In this study water was sampled for 16 highly prescribed pharmaceuticals at 17 sites along the Long Island Sound (LIS) estuary and at the river mouths of four of its major tributaries in Connecticut. Pharmaceutical concentrations were highest in western LIS, ranging from non-detect to 71 ng/L and declined steadily eastward, while river samples ranged from non-detect to 83 ng/L. Two wastewater tracers were tested, sucralose and caffeine, with both accurately predicting pharmaceutical behavior in LIS, while only sucralose performed well at the river sites. Sucralose also correlated well with salinity in LIS, exhibiting conservative behavior along the transect. Attenuation coefficients were determined for measurable pharmaceuticals present in the water column and compared against sucralose to estimate the magnitude of decline in concentrations that may be attributable to in situ degradation. The results provide further evidence of sucralose's effectiveness as a tracer of wastewater-borne contaminants under estuarine conditions.

**MP113 Survey on Chemical Substances used in Daily Life by Target Screening using SPE-LC-Q/TOFMS at Sewage Treatment Plants in Japan**

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For counteracting chemical pollution in water environments, it is necessary to determine the environmental concentration and characterize the generation and discharge of the pollutant itself. However, because many products are used in daily life, it is often difficult to ascertain the origin of some common pollutants. In urban areas, common chemicals collect in sewage treatment plants (STPs). Thus, we focused on determining the amount of generation and discharge of specific chemicals by monitoring at STPs. We monitored a total of 8 STPs in Japan and water samples were collected as 24 h composites of the influent and effluent at each STP in the summer and winter of 2017–2018. In this study, 492 substances were analyzed by target screening method using SPE-LC-Q/TOFMS. Daily generation and discharge units (µg/day/1000 people) were calculated as follows: concentration of substance in the influent (or effluent) (ng/L) × volume of the influent (or effluent) ($m^3$/day) / population (1000 people). A total of 126 substances were detected in the influent and 138 substances in the effluent during the summer survey, and 106 substances in the influent and 118 substances in the effluent during the winter survey. During the both seasons, pharmaceuticals were frequently detected. The total number of the detected substances in the summer survey was greater than that in the winter survey because more pesticides were detected in summer than in winter. The generation units of metformin (diabetes drug), sucralose (artificial sweetener), theophylline (bronchodilator), theobromine, FB351 (UV absorber), and FB71 (fluorescent bleach) were relatively high during both seasons. The generation of theobromine, FB351, and N,N-dimethyl dodecylamine N-oxide (DDAO) was higher in winter than in summer. For metformin and sucralose, the discharge at all STPs were relatively high during both seasons, and their removal rates were relatively low at 67% and 12% in summer, and 71% and 2% in winter, respectively. In both seasons, DDAO showed the largest differences in generation of all detected substances among the STPs. This survey at the STPs using the target screening method with SPE-LC-Q/TOFMS allowed for the clarification of the generation and discharge of pollutants in urban areas, and their removal rates at the STPs.

**MP114 Personal daily discharge unit (DDU) evaluation of various chemicals**

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In this study, we have quantitatively investigated the daily discharge of personal contaminants like pharmaceuticals, cosmetics, fragrances, tobacco smoke, etc. After intake, these chemicals are excreted and released from the human body to a municipal sewage treatment plant (MSTP). We were interested in the average discharge of each chemical through life activities. Therefore, we collected influent water samples from eight sewage treatment plants in Japan. Approximately 1000 substances were analyzed by gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/quadrupole time-of-flight mass spectrometry (LC/QTOF-MS). Approximately 30% and 26% of these analyzed chemicals were detected in the influent, respectively. We then calculated the personal daily discharge unit (DDU) (micrograms/day/1000 persons) for each detected substance, based on the inflow volume and the population associated with each MSTP. To verify the appropriateness of the inflow samples, we calculated the composition patterns of 12 sterols and the percentages of coprostanol and 24-ethylcoprostanol, which are molecular indicators of human feces. The relative contents of coprostanol and 24-ethylcoprostanol were ~21% and 6% in samples collected in summer and autumn, respectively. Furthermore, the average DDU values of coprostanol and 24-ethylcoprostanol were 456 and 125 mg/1000 persons/day at 7 MSTPs, respectively. Approximately 4-times higher DDU values (2012 and 594 mg/1000 persons/day, respectively) were detected at the eighth MSTP, probably due to a large-scale introduction of commuters into the connected area of the eighth MSTP in the daytime. High DDU values were obtained for both fecal sterols and some pharmaceuticals at this MSTP. However, for some chemicals used externally, such as fragrances and dermal pharmaceuticals, no differences in DDU value were observed among the MSTPs, implying that externally used chemicals were discharged mainly by the washing of the body and clothes. Thus, these chemicals were discharged mainly from home bathrooms or laundries rather than office toilets.

**MP115 Estimation of emission amounts of 970 chemicals from human activities in Japan**

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The number and volume of chemicals used daily have been rapidly increasing owing to a greater desire for a comfortable and healthy life. As these chemicals are used in various commercial products, it is essential to understand their emissions and sources to establish measures to reduce their environmental impacts. To estimate the amount of emission and discharge of 970 chemicals into the environment, we examined these chemicals in eight wastewater treatment plants (WWTPs) in major cities in Japan thrice a year by using the Automated Identification and Quantification Database System with gas chromatography-mass spectrometry. Although the total concentrations ranged from 470 to 1600 µg/L, there was no regional difference and seasonal variation in the composition of the chemicals detected in the influents of the eight WWTPs. The dominant chemicals in the influents were sterols (60% of the total concentration), including cholesterol (33%), caffeine (5%), squalene (4%), and L-menthol (2%), which are used in daily life. Further, n-paraffins, which are present in light oil and heavy oil, were detected, indicating the contribution of factories. Since the proportions of the dominant chemicals in the influents were almost the same, their concentrations can be used to estimate the amount emitted in Japan. To confirm this, we compared the amount of di(2-ethylhexyl)phthalate (DEHP) emitted based on calculations using the concentrations with the amount of inflow in WWTPs reported by the Ministry of Economy, Trade and Industry because DEHP is designated as a Pollutant Release and Transfer Register (PRTR) substance. The annually emitted amounts based on the calculations and the PRTR were 200±67 and 118 tons, respectively, which are almost similar. In the case of phenol, which is also designated as a PRTR substance, the annually emitted amounts were 35±31 and 16 tons, respectively, which are also similar like in the case of DEHP. This indicates that this calculation method, which uses concentrations, can be applied to all target substances including those that are not designated as PRTR substances. Nevertheless, the estimated annual discharge of DEHP based on calculations using its concentrations in discharged waters was 35±36 tons, while that reported in the PRTR is 1.2 tons. The gap between these values indicates that further studies are necessary to achieve a more accurate estimation of the amounts of chemicals discharged from WWTPs to the aquatic environment.

**MP116 Discharge scenario of perfluoroalkyl acids into the environment via sewage treatment plants**

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Perfluoroalkyl acids (PFAAs) are detected throughout the world, and thus some of them have been designated as persistent organic pollutants. Although understanding their emission loads and sources are essential to establish measures against their environmental impacts, they are yet to be clarified. PFAAs are used in various commercial products, and therefore small amounts are usually found in wastewater discharged from factories and households, which are typically treated in sewage treatment plants (STPs). Almost the entire volume of wastewater generated due to human activities in urban areas of Japan is treated by STPs. Therefore, we examined PFAAs in STPs located in major cities to determine the amount of PFAAs generated due to human activities. We selected eight STPs (designated as STP A-H) in Japan. Influents and effluents were collected three or four times during the period from February 2017 to February 2018. PFAAs in a 24-h composite sample were extracted by solid-phase extraction, and then measured by LC-MS/MS. The target compounds were 15 different PFAAs (hereinafter, CXA means carboxylic acid type, CXS means sulfonic acid type, and X signifies the carbon number). There were remarkable differences in PFAA concentrations among the STPs examined; PFAA concentrations in the influent and effluent of STP D and those in the effluent from STP G were significantly higher than those of other STPs. The concentrations of PFAA in the influents and effluents were compared. Concentrations of C5A to C9A in effluents were higher than in influents of almost all STPs, while those of C10A to C13A in effluents were lower than in influents. However, perfluoroalkyl sulfonic acid (PFSA) concentrations in effluents were lower than those in influents for all the STPs, except STP G. During water treatment processes, C10A to C13A, and some PFSAs seemed to be removed through adsorption on activated sludge, while C5A to C9A were formed from their parent substances. The loads of PFAA, emitted due to human activities and discharged from the STPs, were calculated using the detected concentrations, volume of wastewater, and population served by the sewer. Based on the median calculated amounts, the amount of PFAA released in Japan was calculated to be 800 kg/y (amount of C8S released was the highest: 210 kg/y), and amount of PFAA discharged from STPs in Japan was calculated to be 810 kg/y (C8S was discharged in the highest quantity: 190 kg/y).

**MP117 Characterization of hexavalent chromium and total chromium in drinking water monitoring data**

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In 2014, the State of California promulgated a maximum contaminant level (MCL) for hexavalent chromium [Cr(VI)] of 10 µg/L, as compared to the MCL for total chromium of 50 µ/L. Although the California MCL for hexavalent chromium is no longer in effect as a result of a court decision, its adoption created immediate interest because hexavalent chromium had been reported in public drinking supplies in California at concentrations exceeding 10 µg/L, but rarely above 50 µg/L. Since that time, a substantial amount of data (over 60,000 samples) on Cr(VI) and total chromium concentrations in public water systems (PWSs) nationwide has been collected as part of the Third Unregulated Contaminant Monitoring Rule (UCMR3). From these data, the Cr(VI) concentration was less than 10 µg/L in approximately 97.6% of groundwater samples and in >99.6% to 100% of other samples (surface water, mixed surface water/groundwater; groundwater directly influenced by surface water). In California, the percentages were approximately 93.9% and >99.2% to 100%, respectively. The total chromium concentration was less than 50 µg/L in >99.8 to 100% of all sample types whether evaluated nationwide or California alone. Compared to the Federal MCL for total chromium of 100 µg/L, 100% of the Cr(VI) concentrations and all but one of the total chromium concentrations were less than 100 µg/L. To evaluate the geographical distribution of higher Cr(VI) and total chromium concentrations, UCMR3 sample locations were estimated for each PWS as the centroid of all ZIP codes that the PWS reported serving (exact locations are not publicly available for security reasons). Using these estimated sample locations, PWSs with higher concentrations of Cr(VI) and total chromium tended to cluster regionally in California, as well as in Arizona and Oklahoma. This analysis could serve as a basis for further investigation of geospatial occurrence or sources of Cr(VI) and total chromium in drinking water.

**MP118 Surface Water Impact from California Cannabis Cultivation**

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Cannabis cultivation may to lead to a substantial increase in pesticide use and subsequent impact to surface water through off-site transport from both indoor and outdoor growing operations. Since January 2018, California's Bureau of Cannabis Control has established residue testing for manufactured cannabis products based on human health concerns. Further investigation is necessary to understand potential environmental impacts. Pesticide use patterns and subsequent off-site transport from cannabis cultivation are poorly understood. The California Department of Pesticide Regulation's Surface Water Protection Program will conduct an environmental monitoring study to evaluate the impacts of legal cannabis cultivation on surface water environments. Site selection will focus on prevalence of grows resulting from local ordinances, cultivation licenses, and water discharge pathways (i.e., wastewater and surface water). Pesticides prioritized for analysis will be selected based on potential for adverse impacts to surface water biological communities and anticipated prevalence in the regulated industry.

**MP119 Optimization of Sampling Design to Determine the Spatial Distributions of Emerging Contaminants in Estuaries**

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Narragansett Bay (NB) is New England's largest estuary and has been the location of numerous multi-disciplinary scientific studies over the last several decades. Most spatial research conducted within NB to date has employed deterministic sampling designs. Several studies have used probabilistic sampling designs on portions of NB; however, a randomized, probabilistic sampling design has seldom been applied to the whole estuary. In this study, a sampling design was developed to implement a probabilistic sampling approach to examine the distribution of emerging contaminants in the estuary. Existing high-density salinity data from the upper portion of NB was used for optimization of the sampling design and results from this analysis were applied to the whole estuary. Five spatial geostatistical interpolation kriging models were compared and the empirical bayesian kriging (EBK) model performed best across the estuary. The results from this analysis were used to determine an optimum number of stations (n=67) and sampling density (0.2 stations/km$^2$) across the estuary. Stations were chosen randomly within a randomized, tessellated hexagonal grid and samples were taken to measure the concentrations of a suite of pharmaceutical compounds. Spatial models using EBK were generated at varying spatial densities from the full number of sampled stations to ¼ the number of stations. Error statistics for each spatial density were generated using cross-validation between predictions and measurements. These statistics were compared using the Derringer desirability function approach. While models were developed for each compound at each spatial density, sucralose was used as a model compound. Results of the desirability comparison indicated that the performance of the spatial model was very accurate at predicting spatial distributions with the full set of samples and that only half of the sites were sufficient to develop a spatial model for NB with only a minor decrease in accuracy compared to the full number of stations. The findings demonstrate the successful use of this geostatistical spatial modeling framework and indicate its potential suitability for use across similar environments.

**MP120 Occurrence of Pyrethroids in Agriculturally Impacted Surface Waters of California**

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The California Department of Pesticide Regulation's (DPR's) Surface Water Protection Program (SWPP) conducts routine monitoring of surface waters impacted by agricultural pesticide applications. Monitoring efforts target high-use agricultural regions guided by DPR's Pesticide Use Reporting (PUR) database and the aquatic toxicity properties of pesticides. Notably, pyrethroids have been detected frequently and at concentrations that exceed the chronic USEPA aquatic life benchmark for invertebrates. For example, the pyrethroids bifenthrin, permethrin, and lambda-cyhalothrin were detected in more than 60%, 30%, and 20% of samples, respectively, between 2011 and 2016 among all monitoring sites in Salinas, Calif. The chronic USEPA aquatic life benchmarks for invertebrates are in the low parts per trillion concentration range for each of these pyrethroids and were exceeded in more than 90% of the samples from this region. Further, the persistence of pyrethroids in sediment contributes to the observed detection frequencies. The water-sediment half-life of bifenthrin is more than six times greater than that of permethrin. Frequent detections and exceedances of toxicity threshold merit a more detailed analysis of pesticide use patterns on commodities with common pyrethroid use in order to suggest effective best management practices or other mitigation measures.

**MP121 Distribution and Toxic Potential of Pyrethroids, Alkylphenols, and Nonylphenol Ethoxylates in Minnesota Lake Sediments**

*J.L. Crane, Minnesota Pollution Control Agency / Environmental Analysis & Outcomes Division*

Pyrethroids are the third most applied group of insecticides worldwide and are used for both agricultural and urban applications. These compounds are of concern because they are extremely toxic to fish and non-target benthic invertebrates, and this toxicity increases with colder water temperatures. Bifenthrin and permethrin are the major pyrethroid compounds sold in Minnesota, and most of the bifenthrin sales are for crop chemicals. Alkylphenols (APs) are breakdown products of high production alkylphenol polyethoxylates like nonylphenol ethoxylates (NPEs). These compounds are of concern due to their toxicity, estrogenic properties, and widespread environmental contamination. Sediment data on the distribution of these compounds in Minnesota's waterways are sparse. To this end, a project was conducted to address the broad management question of "What are the environmental impacts of these contaminants in Minnesota lake sediments?" This study capitalized on the random sampling design, field sampling assistance, and logistical resources of the USEPA's National Lakes Assessment (NLA) project during the summer of 2017. Thirty of the 50 Minnesota NLA lakes selected for this study targeted lakes in agricultural watersheds, plus lakes in other major land use groups to provide broad geographic coverage. Surficial sediment samples (0-5 cm) were collected from the deepest area of each lake, composited, and split from other samples utilized for NLA conventional analytical parameters. The Minnesota Pollution Control Agency funded the analyses of pyrethroids (and pyrethrins), APs, and NPEs by SGS AXYS Analytical Services Ltd. Several samples had challenging matrix interferences that increased the reporting limits. The Kaplan-Meier means were 12.3 ng/g dw for 4-nonylphenol (53.3% NDs), 11.5 ng/g dw for 4-nonylphenol monoethoxylates (73.3% NDs), and 1.5 ng/g dw for bifenthrin (73.3% NDs). Resmethrin and 4-nonylphenol diethoxylates were each detected in one lake. The other pyrethroids and 4-n-octylphenol were not detected in any of the lakes. Specific GIS-watershed data are currently being obtained. The results will be used to calculate pyrethroid Toxic Units for benthic invertebrates, to make comparisons to available sediment quality guidelines, to possibly compare results of 4-nonylphenol by major watershed land uses, to calculate background threshold values for data with < 80% nondetects, and to compare the results with data from other North American sediments.

**MP122 Pesticides in Washington State Sediments**

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The Washington State Department of Agriculture (WSDA) has been actively conducting surface water monitoring for pesticides in Washington State since 2003. In 2015 staff developed a pilot study to monitor for pesticides in in streambed sediments with the goal of assessing the need for more routine sediment monitoring by estimating the potential risks to benthic invertebrates from exposure to the pesticides that were found in the stream bed sediments. Stream bed sediments were collected during three separate sampling events at five of the regular WSDA surface water monitoring locations. Thanks to ongoing work being conducted by a collection of municipal stormwater permitees who collaborate under the western Washington municipal stormwater permit, stream bed sediments were also collected at an additional 81 sites around the greater Puget Sound region. The land use characteristics in the watersheds where the sampling locations were located varied between dominantly agriculture and dominantly urban. Each of the 93 samples were analyzed for 126 pesticide compounds and total organic carbon (TOC). All samples collected between March and October of 2015, the typical pesticide application season. There were a total of 45 detections of 12 unique compounds. The most frequently detected compounds were DDT, DDT degradates and bifenthrin. TOC normalized concentrations were compared to previously published Likely Effect Benchmarks (LEB) to assess toxicity to benthic invertebrates. Bifenthrin is an insecticide that is commonly found in household and agricultural products and was the most commonly detected pesticide. In 81% of the samples containing bifenthrin its TOC normalized concentration was found to be above the LEB, indicating high probability of adverse effects on benthic invertebrates. The results of the study indicate that further investigation is warranted due to the presence of pesticides in streambed sediments at levels that may affect benthic invertebrate communities. Plans to resume and possibly expand sediment monitoring for pesticides are planned following laboratory method development that is being implemented to combat the low detection frequencies and high reporting limits encountered in this study.

**MP123 Quantification of pharmaceuticals, personal care products, and perfluoroalkyl substances in sediments from two urban bays, Puget Sound, WA**

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Pharmaceuticals and personal care products (PPCPs) and perfluoroalkyl substances (PFASs) are identified as chemicals of emerging concern (CECs) in Puget Sound, WA, due to their potential to cause adverse toxicological, biological, and ecological effects when unintentionally discharged to the environment. Characterization of sources, transport patterns, and the fate of CECs in the environment has been prioritized as part of the toxics monitoring strategy for the Sound. To this end, concentrations of PPCPs and PFASs have been measured and detected in influent, effluent, and biosolids from municipal wastewater treatment plants; in the tissue of invertebrates, fish, birds, and marine mammals; and in surface sediments from ten long-term monitoring stations and from stations in Bellingham Bay and Elliott Bay. Continuing the series of assessments in Puget Sound's urban bays, sediments were tested for the presence of 119 PPCPs and 13 PFASs in Commencement Bay in 2014 and in Bainbridge Basin in 2015. Analyses were conducted for PPCPs and PFASs by AXYS Analytical Services Ltd., Sidney, BC, Canada, using AXYS Method MLA-075, based on EPA 1694 and MLA-041, respectively. These methods use liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS). Results are summarized and presented graphically to indicate the concentration and distribution of PPCPs and PFASs in these Puget Sound urban sediments, with comparisons made to concentrations measured in previous Puget Sound surveys.

**MP124 Presence and sources of drugs, steroids and xenoestrogens in water and sediments from the tidal freshwater Potomac River**

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Selected micropollutants, including endocrine modulators, illicit drugs, therapeutic drugs, detergents, sun screen agents, and parabens were measured in water and riverbed sediments (2017 and 2018) in a tributary embayment of the tidal freshwater Potomac River (Hunting Creek, Alexandria, VA, USA) in the vicinity of an urban wastewater treatment plant (WTP). The aquatic samples were analyzed by using solid phase extraction (water) and QuEChERS (sediment) in combination with liquid chromatography/tandem mass spectrometry. The micropollutants were detected in water at concentrations ranging from 450 to 6 ng/L. Steroid hormones, trimethoprim, and dextromethropham were the most frequently detected constituents in water. In sediment, micropollutant concentrations ranged from 350 to 8 ng/g dwt, with progesterone, dextromethorpham, bisphenol A and prednisone being the most abundant. The geospatial distribution of the micropollutants in Hunting Creek indicated the steroids correlated with spatial proximity to WTP discharge in water and sediments, but the proximity associations were tentative in Hunting Creek given the complex nature of urban emissions and hydrodynamics of a large urban tidal river. Source apportionment using principal components analysis coupled with partial least squares was used to quantify the importance of several identified emission sources. There were multiple sources that could be attributed to the micropollutants found in Hunting Creek, most notably combined sewer outfall discharge and WTP effluent. Apportionment results showed that emission sources for micropollutants can be difficult to identify in large rivers adjacent urban centers where a wide variety of potential emission sources exist.

**MP125 Documenting Ambient PAH Concentrations in California**

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Natural Resource Trustees (California Department of Fish and Wildlife, Office of Spill Prevention and Response, the National Oceanographic & Atmospheric Administration, the US Fish & Wildlife Service, and the Bureau of Land Management) in conjunction with Chevron, have jointly completed Ephemeral Data Collection Plans (EDCPs) for Chevron's marine terminal in Eureka, lightering station near San Diego, and refineries in El Segundo and Richmond, California. Each EDCP includes sampling protocols for environmental media (e.g., water, sediment, and tissue), pre-determined sampling sites, and contact information for first responders, consultants, and laboratories. Samples were collected near the Eureka Terminal and El Segundo Refinery over multiple years and ambient concentrations of polycyclic aromatic hydrocarbons (PAHs) were measured using modified EPA methods. Results show PAH profiles and concentrations vary between sampling years at each site. This is likely due to environmental factors including tidal changes, weather, land use, and petroleum release that are in constant fluctuation and can influence detections overtime. Results also show that PAH profiles and concentrations vary between sampling media. Sediment and tissue samples had measurable concentrations of PAHs such as phenanthrene, flouranthene, and pyrene, whereas PAHs in water samples were often not detected. PAHs in sediment and tissues are often more representative of ambient concentrations due to their affinity to adsorb and bioaccumulate PAHs. Whereas PAH detections in water are often sporadic due to their low water solubility and high affinity for particulate matter. Chemistry results of collected samples has provided valuable ambient data and continual monitoring will provide a better understanding of PAH concentrations at pre-determined sites. This data will help establish pre-spill reference conditions in the event of a petroleum release near or at the sampled sites.

**MP126 Production and Certification of a New Sediment Reference Material: Standard Reference Material 1936 Great Lakes Sediment**

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The National Institute of Standards and Technology provides many types of natural matrix Standard Reference Materials (SRMs) certified for organic and inorganic contaminants. The materials can be used by laboratories as control samples, to validate methods, assign values to in-house control materials or to measure new contaminants without certified values. SRMs are available for many different matrices including soil, fish, mussel tissue, air particulate matter, house dust, sewage sludge, human blood and sediment. NIST currently has two estuarine sediment materials: SRM 1944 New York/New Jersey Waterway Sediment and SRM 1941b Organics in Marine Sediment. The materials have been certified for polychlorinated biphenyl congeners (PCBs), polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ether congeners (PBDEs), chlorinated pesticides, perfluorinated alkyl substances (PFAS) and trace elements. SRM 1936 is being produced to augment this suite of sediments by providing a contemporary fresh water material from an urbanized location in Great Lakes. SRMs 1941b and 1944 are higher in sulfur and were collected over 20 years ago. For SRM 1936, 480 kg of surficial sediment was collected from Milwaukee Harbor, air dried, milled, radiation sterilized and then bottled. The material will be certified for PAHs, trace elements and value assigned for a variety of other organic contaminants including PCBs, PBDEs and PFAS.

**MP127 Release and Transport of Soluble Phosphate-P from Sediments, Soils and Senescent Leaf Fragments in the W. Branch Brandywine Creek, PA**

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Phosphorus is a key nutrient for plant growth but too much of it can trigger unwanted algal growth in waterways. The W. Branch Brandywine Creek in Chester County, PA is plagued by excessive P levels, causing it to fail to meet in-stream water quality standards established by the Clean Water Act of 1972. Phosphorus tends to adhere to solid sediments but evidence suggests that it can be released back to aquatic systems following sediment re-suspension during storm events. This study examines P release kinetics from associated sediments, soils and plant tissue (leaves). Our results from leaching studies for an air-dried, sieved (< 1.0 mm) sediment sample obtained from the site of an old ironworks dam in Hibernia County Park (40.029446°N, 75.835647°W) show that P release follows a two-stage mechanism; fast release occurs over the first few hours of mixing with lab water followed by a slower release mechanism over the next several days. Modeling followed the relationship: Soluble $[PO_4\text{-}P] = C_{Fast}*[1-e^{(-k_{Fast}*t)}] + C_{Slow}*[1-e^{(-K_{Slow}*t)}]$; where C = maximal concentration of fast and slow releases, k = first order rate coefficients for fast and slow release and t = time; model fits showed $C_{Fast} = 0.069$ mg/L, $k_{Fast} = 1.259$ day$^{-1}$, $C_{Slow} = 0.341$ mg/L and $k_{Slow} = 0.002$ day$^{-1}$. Similar results have been obtained for sediments from other creek locations. Sieved (< 1.0 mm) soils from the old ironworks pond basin (now dry; the dam at this site was breached sometime after 1937) were collected at 40, 101, 172 and 203 cm deep, corresponding to different deposition times pre-1940s. These soils failed to show significant P release even after 11 days of mixing. Senescent Sugar Maple (*Acer saccharum*) and American Sycamore (*Platanus occidentalis*) leaves just prior to leaf drop in October were collected, dried at 105°C, crushed and sieved (< 2.0 mm) then added to lab water; mixing for 48 hours showed significant release of P, up to 2.5 mg/L for Maple and 4.7 mg/L for Sycamore after just 24 hours. While these results illustrate P release kinetics from historical sediments and those deposited in the more near term, ongoing work is examining release from fresh, storm-associated sediments. These combined results will help elucidate the mechanisms of phosphorus deposition and transport and can be used to plan for control and cleanup efforts.

**MP128 Monitoring of Endocrine Disrupting Pesticide Residues in Soils in Korea**

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To evaluate residues of seventeen pesticides suspected as endocrine disruptor in agro-environment, total four hundred and eighty soils were sampled in nationwide arable land except paddy field on March and April 2000-2002. One hundred and sixty soil samples were collected in green houses cultivated six fruit vegetables (tomato, strawberry, melon, watermelon, cucumber and pepper), leafy perilla and lettuce in 2000. On hundred and seventy soil samples were collected in fields mainly cultivated red pepper, soybean, sesame, perilla, potato, sweet potato and tobacco in 2001. And one hundred and fifty soil samples were collected in orchards cultivated apple, pear, grape, citrus, sweet persimmon and peach in 2002. Carbendazim, mancozeb and vinclozolin as fungicides were detected in two hundred and seventy four, one hundred and sixty three and twelve samples, respectively and their average residue concentrations in positive samples were 0.044 $mgkg^{-1}$ for carbendazim, 0.031 $mgkg^{-1}$ for mancozeb and 0.018 $mgkg^{-1}$ for vinclozolin. Endosulfan, an organochlorine insecticide, was detected in four hundred and forty one samples, and its average residue concentrations in positive samples were 0.25 $mgkg^{-1}$ for greenhouse soils, 0.16 $mgkg^{-1}$ for upland soils and 0.082 $mgkg^{-1}$ for orchard soils. Dicofol, another organochlorine insecticide analyzed, was detected 0.25 $mgkg^{-1}$ of average residue concentration in positive samples in eleven soils. Cypermethrin and fenvalerate as pyrethroid insecticide were detected in four and twelve samples, respectively and their average residue concentrations in positive samples were 0.059 $mgkg^{-1}$ for cypermethrin, 0.013 $mgkg^{-1}$ for fenvalerate. Parathion, an organophosphorus insecticide, was detected in twenty eight samples and its average residue concentration in positive samples was 0.107 $mgkg^{-1}$. Except some outliers such as parathion-applied at near sampling time, average residue concentration was 0.020 $mgkg^{-1}$. Malathion, another organophosphorus insecticide analyzed, was detected 0.006 $mgkg^{-1}$ in one soil samples cultivated garlic. Methomyl, a carbamate insecticide, was also detected 0.066 $mgkg^{-1}$ in one greenhouse soil cultivated lettuce. Carbaryl, another carbamate insecticide analyzed, was not detected at any soil samples. Among four herbicides, alachlor was detected in forty six samples and its average residue concentration in positive samples was 0.041 $mgkg^{-1}$, but metribuzin, and trifluralin were not detected at any soil samples.

**MP129 Background Threshold Values – ProUCL Guidance and Technical Issues (USL, UPL, UTLs)**

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USEPA's ProUCL statistical package has revolutionized the determination of background threshold values (BTVs) by offering a myriad of options and methods. Unfortunately, guidance on which exact method to use in any particular circumstance is not clearly presented in a single place in the manuals. We will review the types of BTVs available in ProUCL (UTL, UPL, USL), their assumptions and requirements (e.g., no outliers, single population, representativeness, and sample size), and limitations of each type (multiple comparison failure rates, "brittleness" to minor assumption violations, result reproducibility). Also, there will be an overview of how ProUCL can treat non-detect values. A summary table by method including limitations and suggestions will be included based on lessons learned.

**MP130 Applications of NMR Spectroscopy in the Characterization of Contaminated Environments**

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Nuclear magnetic resonance spectroscopy is a primary analytical tool for the elucidation of unknown chemical structures, however its application in the area of environmental chemistry is relatively limited. This is particularly true with respect to the practical application of analytical chemistry in the assessment and management of contaminated environments. In general, conventional approaches for environmental analysis are based on the targeted identification and quantification of known or presumed environmental contaminants. This approach is useful for the assessment of most environments relative to regulatory guidelines, however traditional approaches often provide incomplete characterization of environments that are either heavily contaminated or that are contaminated with unknown compounds for which standardized methods have not yet been established. This poster will discuss the application of NMR spectroscopy as a powerful non-targeted analytical tool for the elucidation of unknown contaminants present at heavily contaminated environments. While NMR is not as sensitive as other detection methods, its ability to provide unbiased and unambiguous structural information on unknown constituents can help improve the characterization of the contaminants present at heavily contaminated environments for which conventional analytical tools have been able to provide only partial characterization.

**MP131 Analysis of Artificial Sweeteners by Hydrophilic Interaction Chromatography**

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Artificial sweeteners (ASs) including acesulfame (ACE), aspartame (APM), and sucralose (SUC) provide sweetness without adding calories. ASs are widely used as sugar substitutes in foods, drinks, drugs, and sanitary products. Some ASs are excreted unchanged from the human body, and flow down into waste water treatment plants through sewer. ASs having hydrophilic and stable properties are unremoved through waste water treatment, and are discharged into the environment. Recently, environmental occurrence, fate, and ecotoxicological effect of ASs have been investigated in the world. Especially, the two artificial sweeteners ACE and SUC have been found ranged up to 10 µg/L in the water environment. The high aqueous concentrations of ACE and SUC combined with their persistence, high water solubility, and low absorbability to solids also make them virtually ideal anthropogenic wastewater markers. The determination of ASs were usually carried out by solid-phase extraction (SPE) combined with liquid chromatography (LC) /tandem mass spectrometry (MS/MS). However, it was reported that the response of SUC in LC/MS/MS was much lower than those of other ASs. To overcome this problem, in this study, a simultaneous analysis of ASs including SUC by hydrophilic interaction chromatography (HILIC) using a polymer-based column was developed. The target ASs were ACE, APM, SUC, cyclamate (CYC), neotame (NTM), saccharin (SAC). The ASs were analyzed using a polymer-based column (HILICpak VG-50 2D, 150 mm × 2.0 mm i.d., 5 µm particle size; Shodex) and Xevo TQD coupled with ACQUITY UPLC (Waters). The polymer-based column having wide range pH stability is suitable for basic mobile phases. Effects of mobile phase on AS intensity were tested: 0.02 % formic acid, 5 mM ammonium formate, 5 mM ammonium formate (pH 9.5), or 0.1 % ammonium hydroxide as aqueous mobile phase; acetonitrile as organic phase. High-intensity peaks of AS were obtained by the combination of acetonitrile and 0.1 % ammonium hydroxide. The ASs were also good separated by HILIC gradient elution using acetonitrile and 0.1 % ammonium hydroxide solution. Instrumental detection limits ranged from 0.2 ng/mL (ACE) to 0.6 ng/mL (APM). Furthermore, it was confirmed that the HILIC method was applicable to determination of ASs in river water and ground water.

**MP132 Analysis of Volatile and Semi-Volatiles using Thermal Desorption and application to Two Passive-sampling Matrixes**

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This work describes the development of the thermal desorption extraction process using a Markes Micro-Chamber/Thermal Extractor (M-CTE 250) and the analytical method to examine 35 volatile organic compounds (VOCs) including BTEX and alkanes, 6 Tri-alkyl/phenyl-organophosphates (Tri-R's), 8 oxygenated polycyclic aromatic hydrocarbons (OPAHs), and 28 polycyclic aromatic hydrocarbons (PAHs). This methodology dramatically reduces the use of organic solvent and preforms this analysis in a single quantitative run using a Markes thermal desorption system interfaced with an Agilent 6890N gas chromatograph / 5975B mass spectrometer (GC/MS). Volatile organic compounds had an average detection limit (DL) of 1.0ng, Tri-R-phosphates had a DL of 3.0ng, OPAH's had a DL of 1.5ng, and PAHs had a DL of 1.1ng with a run time of 31.13mins. Analytic method validation included standard spiked wristband passive samplers and artificial turf analyzed in replicates of three on three different days. VOCs had an average recovery of 89.3% and 5.1% relative standard deviation (RSD), Tri-Rs had a 105.1% recovery with a 13.7% RSD, OPAHs 65.5% recovery with a 9.3% RSD, and PAHs a 94.1% recovery and a 7.2% RSD.

**MP133 PFAS analysis in surface water using Agilent Ultivo triple quadrupole LC/MS with ASTM D7979 with the addition of Gen-X and Adona**

*E. Parry, Agilent Technologies; T. Anumol, Agilent*

Per/Polyfluoroalkyl substances (PFAS) are chemicals found in firefighting foams in consumer products requiring water resistant and stain repellant properties. The USEPA has made addressing PFAS water contamination a national priority, due to their presence in US drinking water sources. EPA method 537, specific to drinking water, provides a direct injection method for 14 PFAS compounds. For other types of water samples, ASTM D7979 provides a simple extraction and analytical method for 21 PFAS compounds. Currently, new fluorochemicals such as GenX and Adona are being identified in the environment and classified as problematic. The Agilent Ultivo is a stackable triple quadrupole liquid chromatogram mass spectrometer (LC/MS). We demonstrate the robust analytical performance of Gen X, Adona, and ASTM D7979 targets in surface water. Minimal method modification was required to accommodate the additional analytes.

**MP134 Contamination Analysis of the NIST Marine ESB's Post-Homogenization Cleaning Protocols**

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The National Institute of Standards and Technology's (NIST) Marine Environmental Specimen Bank (Marine ESB) provides researchers with homogenized tissues to be analyzed for a variety of biological and chemical indices. Tissues are cryohomogenized using polytetrafluoroethylene (PTFE) disk mills specifically designed for the process and tissue homogenates are aliquoted into perfluoroalkoxy alkane (PFA) jars. While the PFA jars come cleaned directly from the manufacturer and have not previously come into contact with biological tissues, the PTFE disk mills are custom made and expensive so consequently are reused. Therefore, after cryohomogenization is complete, dirty disk mills are washed according to Marine ESB protocols. Although this has been standard practice since the inception of the Marine ESB, little was known about potential carry-over of contaminants from sample to sample via cleaned and reused PTFE disk mills. Therefore, an investigation was conducted to evaluate the effectiveness of eliminating compounds commonly assessed in Marine ESB samples (i.e., organic and inorganic analytes) using the established cleaning protocol. Long-finned pilot whale (*Globicephala melas*) blubber and pygmy sperm whale (*Kogia breviceps*) liver samples were cryohomogenized and aliquoted into pre-cleaned PFA jars. The used PTFE disk mills were cleaned according to Marine ESB protocols. Once dry, the PTFE disk mills were rinsed three times with analysis-appropriate solvents in order to extract any remaining analytes, which could result in carry-over contamination. Homogenates and disk mill rinsates were analyzed in NIST's Analytical Chemistry Labs. Organic analytes (i.e., polychlorinated biphenyls (PCB) and organochlorine contaminants (OC)) were analyzed using gas chromatography-mass spectrometry (GC/MS). Inorganic analytes (i.e., trace elements) were analyzed using inductively coupled plasma mass spectrometry (ICP-MS). Results from this study indicate the cleaning protocol established by the Marine ESB sufficiently removes organic and inorganic analytes from used equipment with minimal carry-over between samples as a result of the post-homogenization cleaning procedure.

**MP135 Novel in vivo sampling probe based on water-absorption for ultrasensitive neonicotinoids determination in vegetable**

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Neonicotinoids represent the fastest growing class of pesticides, of which imidacloprid is the most used worldwide. However, due to the unique physical-chemical properties of neonicotinoids, sampling, especially in vivo sampling in plant tissues, represents a formidable challenging. In this study, we developed a functionalized solid-phase microextraction (SPME) probe with a strong capability for water absorption and tested it for direct in vivo sampling of neonicotinoids in vegetable tissues. The coating of the SPME probe possessed both water absorption and bioinspired antibiofouling properties. After sampling, the SPME fiber was retrieved, freeze-dried, and directly desorbed into a UPLC-MS/MS system for quantification. The coating was demonstrated to be much more superior to the commercial SPME fiber coatings. The method showed outstanding extraction capacity and efficiency toward neonicotinoids, and high sensitivity as well as reproducibility , suggesting a great applicability for in vivo application in live plants. This novel sampling technique opens up new avenues for the facile and efficient in vivo sampling of neonicotinoids in plant tissues and may be used to improve our understanding of ecotoxicological effects of this important class of insecticides, including their deleterious effects on pollinators.

**MP136 Method for the Determination of Drugs and Pesticides in Catfish Feed for Contaminant Traceback**

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To ensure the safety of the food supply in the US, many organizations are working to develop and improve testing methods. In cooperation with the USDA/FSIS/FERN, the Mississippi State Chemical Laboratory (MSCL) has developed a method for the determination of drugs and poisons in catfish feed using GC/MS/MS and LC/MS/MS analysis. A surveillance program within the state supports the growth of the catfish industry in Mississippi and is based upon generating a product that tastes good and is safe for all consumers. The MSCL analyzes multiple compounds (currently-used pesticides and metabolites) in farm-raised catfish, and participates in TOX 1 screening of catfish for the USDA/FSIS/FERN. The feed method analytes include: acephate, aldrin, arecoline, atrazine, azoxystrobin, bifenthrin, chlorpyrifos, clomazone, chloransulam-methyl, codeine, cyfluthrin, cyhalothrin, cypermethrin, DDE, DDT, deltamethrin, diazinon, dicofol, dicrotophos, diuron and metabolites (DCPMU, DCPU), endrin, fentanyl, fenvalerate, flonicamid, flumetsulam, formesafen, imidacloprid, malathion, myclobutanil, nicotine, paraxoon, parathion, pentazocine, phorate, pilocarpine, profenofos, propiconazole, pyraclostrobin, scopolamine, strychnine, terbuphos, tetramine, thiamethoxam, and tribufos. An Agilent 7890A/7000 Triple Quad MS equipped with a HP5MS and an Agilent 1290 LC/6460 Triple Quad MS equipped to an Eclipse Plus C18 are used for the analysis of the compounds. The method includes extraction of fish feed in water (two shakes, one basic and one acidic), acetonitrile, and QuEChERs packet (AOAC), followed by centrifugation. The extracts have various matrix effects depending upon the type of feed. The addition of an alumina SPE column coupled to a C18 SPE column decreases the matrix effects while maintaining acceptable recovery rates for most compounds in a screening method. The columns are eluted with 0.1% formic acid in acetonitrile. Recovery of most compounds are greater than 60%, except pentazocine – 53%, scopolamine – 56%, strychnine – 40%, codeine – 36%, arecoline – 33%, and nicotine – 0%. The drug and pesticide catfish feed method has added an additional tool to the laboratory to help to identify contaminants in catfish feeds that may adulterate the edible catfish fillets when fed to fish. This method will increase the capacity of USDA's surveillance sampling and provide the ability to test a potential source of contamination.

**MP137 Urinary biomarker discovery of toxicants using mass spectrometry-based metabolomics data processing approaches**

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Toxicant metabolites are taken as biomarkers for human toxicant exposure assessments. Metabolism within an organism is extremely complex. To detect toxicant metabolites from such a complex matrix is challenging. Mass spectrometry-based metabolomics data processing approaches have emerged as an ideal method of exposure marker identification. Ultra-performance liquid chromatography coupled with Orbitrap high-resolution mass spectrometry (MS) and two data processing approaches, the signal mining algorithm with isotope tracing (SMAIT) and the mass defect filter (MDF), were used for toxicant exposure biomarker discovery. Di-(2-propylheptyl) phthalate (DPHP) was taken an example toxicant. DPHP is a plasticizer used in polyvinyl chloride and vinyl chloride copolymer that has been suggested to be a toxicant in rats and may affect human health. Because the use of DPHP is increasing, the general German population is being exposed to DPHP. To date, the knowledge regarding DPHP metabolism has been limited, and only four metabolites have been validated in human urine. Our results show 13 and 104 metabolite candidates were identified by the MDF and SMAIT, respectively, in in vitro DPHP incubation samples. Of these candidates, 17 were validated as tentative exposure biomarkers using a rat model, 13 of which have not been previously reported. The two approaches generated rather different DPHP exposure biomarkers, indicating that these approaches are complementary for discovering exposure biomarkers. Compared with the four previously reported DPHP metabolites, the three novel biomarkers had higher peak abundances, and two of them were confirmed as DPHP structure-related metabolites based on their MS/MS product ion profiles. The mass spectrometry-based metabolomics data processing approaches were successfully applied to identify toxicant exposure biomarkers.

**MP138 Development of a multi-mycotoxin analysis method in wine by LC-MS/MS featuring a comparative extraction study**

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Studies concerning the contamination of Wine by mycotoxins such as Ochratoxin A and the Fumonisins during the process of producing wine have been widely documented. Fusarium mycotoxins and Aflatoxin are not usually detected or reported in wine, however this is not confirmation that they do not contaminate wines as various factors including climate change have increased concern for the contamination of wine by these mycotoxins. Methods that do include multi-mycotoxin analysis, usually employ classical extraction techniques such as liquid–liquid extraction (LLE) or solid–phase extraction (SPE), which include several disadvantages such as large sample volumes, extensive amounts of hazardous solvents, and time-consuming sample preparation. This study compared a variety of extraction methods aimed at lowering sample extraction time, decreasing cost, and lowering solvent/sample usage while retaining simplicity. A salting-out liquid–liquid extraction (SALLE), modified QuEChERS (quick, easy, cheap, effective, rugged, and safe) method, and Dispersive liquid–liquid microextraction (DLLME) method where employed for the quantitative analysis of 14 different mycotoxins (Zearalenone, Ochratoxin A, Patulin, Deoxynivalenol, Nivalenol, AFB1, AFG1, FB1, FB2, Tenuazonic Acid, Alternariol, Citrinin, T-2, HT-2) by LC-MS/MS to determine the occurrence of these toxins in imported and domestic wine samples.

**MP139 Method Validation for the Determination of Herbicide "X" in Larval and Adult Bee Diet**

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The honey bee *(Apis mellifera)* is an important pollinator for a variety of agricultural crops. Its importance in agricultural success has caused investigators to use the honey bee as a test species for the evaluation of the effects of agricultural chemicals on non-target insects. This is in accordance with the guidelines set by the Environmental Protection Agency (EPA) for the registration of chemical substances whose regulations require a risk assessment on the potential harm of chemical substances to humans, wildlife, and non-target organisms. In order to support the toxicity test of herbicide "X" on the honey bee, an analytical method was developed and validated to determine the amount of herbicide "X" in larval diet and adult bee diet (sugar water), the diet being the means of exposure to the honey bee. Larval diet samples were prepared using appropriate stock solutions in acetone. Due to poor recoveries the solubility of herbicide "X" in sugar water was investigated through a series of trials in order to determine the most effective method of introducing herbicide "X" to the matrix. The composition of the sugar water (50% versus 67% sugar water) and fortification method (fortification using stock solution versus formulation) was investigated. The method yielding the highest percent recoveries used 67% sugar water and fortification with formulation of herbicide "X". The method was validated through a series of matrix blanks and fortifications. The matrix fortification for the larval diet was performed in quintuplicate at 40.9 and 1636 mg a.i./L and the sugar water performed in quintuplicate at 300 and 4500 mg a.i./kg. Samples were processed through a series of dilutions which included matrix matching. Matrix fortifications were analyzed against standards prepared from the reference substance also made in matrix matched solution. Matrix fortifications were analyzed using HPLC with ultraviolet detection. The compound's percent recovery was calculated using a combination of OpenLab and Microsoft Excel 2010. Mean percent recovery at the low concentration level for larval diet was 96.8% and the high level was 86.7% with an overall precision of 6.65%RSD. Mean percent recovery at the low concentration level for sugar water was 114% and the high level was 114% with an overall precision of 7.54%RSD. Validation results suggest herbicide "X" in larval diet and sugar water can be accurately determined using the analytical method.

**MP140 Simultaneous determination of neurotransmitters and their precursors /metabolites in animal brain tissue**

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Endogenous neurotransmitters play a critical role in function of central nervous system (e.g., activity, attention, emotion). Some in vivo studies have been reported alterations in the brain levels of several neurotransmitters following exposure to environmental pollutants such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs). However, the mechanisms of neuroendocrine disruption are still unclear, and there has been an increasing demand for quantification of neurotransmitters and their precursors/metabolites in biological matrices. The present study aimed to develop an analytical method for 14 neurochemicals of not only neurotransmitters but also precursors and metabolites in animal brain tissues by using ultra-fast liquid chromatography-tandem mass spectrometry (UFLC–MS/MS). Protein precipitation with acetonitrile was chosen for cleanup because the process reduced signal suppression for target compounds, and thus, protein precipitation. To evaluate extraction efficiency and recovery, 3 different types of extraction solvents, which were methanol/Milli-Q water (1:1, v/v) (1) non-acidified, (2) acidified with acetic acid and (3) acidified with formic acid, were tested. When using the solvent acidified with formic acid, the highest extraction efficiency was observed for dopamine, L-Dopa and 3-MT. On the other hand, low recovery rates of norepinephrine and octopamine were found. Thus, the solvent acidified with formic acid was adopted for analysis of dopamine, L-Dopa and 3-MT, while non-acidified solvent was used for extraction of the other compounds. In this analytical condition, acceptable recovery rates (71%–120%) and intra- and inter-day precision (CV: < 7% and < 10%, respectively) were obtained. The limits of quantification, which were estimated as the concentrations giving a peak with a signal-to-noise ratio of 10 in brain extracts, ranged from 3.4 to 330 ng g-1 wet weight for the target neurochemicals. These values were comparable to or lower than those reported in previous studies. The developed method was finally applied to brain tissues of 3 beagle dogs, and 4 neurotransmitters and 4 precursors/metabolites were successfully quantified. Our UFLC–MS/MS method allows the exploration of the profile changes of neurotransmitters caused by exposure to environmental contaminants such as PCBs and PBDEs.

**MP141 Nanosecond pulsed electric field incorporation technique to predict toxicity of equine estrogens on medaka embryos**

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Using a nanosecond pulsed electric field (nsPEF) technique, we have assessed teratogenicity and embryonic developmental toxicity of equine estrogens (Eqs) and also tried to predict the molecular mechanisms of teratogenicity and embryonic developmental defects caused by Eqs on medaka (Oryzias latipes). The 4-5-h post-fertilization embryos were exposed to co-treatment with 0.1, 1, 10 ppm Eqs (equilin:Eq and equilenin:Eqn ) and nsPEF and then continuously cultured until hatching. Results documented that the time to hatching of embryos which exposed with 1ppm of both Eq and Eqn by nsPEF groups was significantly delayed in comparison to the control group. In addition, an abnormal embryo development such as the severe dysfunction, the delay of blood vessel formation and teratogenicity (abnormal bone formation) was observed in the surviving medaka eggs from 10 ppm of both Eq and Eqn exposure using nsPEF. The nsPEF incorporation technique can import Eqs within very narrow time windows in the early embryonic stages. From the results in this study, we indicated that our nsPEF technique become a powerful tool for assessing teratogenicity and embryonic developmental toxicity of chemicals, and also enable to predict their molecular mechanisms in medaka embryos.

**MP142 Novel High Through-Put Screening of Transition Metal-Based Anticancer Compounds Using Zebrafish Embryo Larval Assay and ICPMS Analysis**

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The use of the zebrafish embryo larval assay (ZELA) as a whole organism high throughput screening method for potential anticancer drug candidates coupled with ICPMS has proven to be a valuable research tool. This bioanalytical assessment of delivered tissue doses allows for expedited screening of potency and idiopathic off-target effects. Additionally, the zebrafish model allows for examining adverse outcome pathways from biochemical to whole organism endpoints, which are difficult to do in cell culture and cost limiting using rodent models. ZELA is especially relevant to the success and prolific development of metal-based compounds. The ability to screen large numbers of these anticancer compounds makes ZELA desirable. We hypothesize that the zebrafish model coupled with ICPMS could be used to evaluate potency, off-target toxicity, efficacy, and tissue dose. As an initial proof of principle toxicity studies using cisplatin were carried out. An embryo digestion and ICPMS method was developed for the detection of Pt and was found that for following waterborne exposure concentrations of 0, 3.75, 7.5, 15, 30 and 60 mg/L, the respective doses were 0.05, 8.7, 23.5, 59.9, 193.2, and 461.9 ng of Pt per embryo (Pt/E). Dose response curves for lethality and a non-lethal delayed hatching endpoint were calculated. The lethal endpoints were determined: LC50: 31 mg/L for waterborne exposure, LD50: 193.2 Pt/E for quantified delivered dose. For the delayed hatching endpoint, the LOAEL for waterborne exposure was 3.75 mg/L and an associated delivered dose of 8.7 Pt/E; and an EC50 of 4.6 mg/L. A proprietary Ru-based molecule, LCR134, was also evaluated and resulted in an LC50 of 4.4 µM, EC50 of 3.5 µM, NOAEL of 2 µM and lethal tissue dose of 3 significant differences in total body length, intraocular distance, pericardial and yolk sac size were observed. Additionally, several organ systems had lesions and the underlying mechanisms will be examined to better understand potential side effects that may be observed in rodent or human drug trials. This research was funded by NJAES-Rutgers NJ01201, NIH-MIEHS P30 ES005022, and Training Grant T32-ES 007148, FCT (project UID/QUI/00100/2013), Valente, A. acknowledges the Investigator FCT2013 Initiative for the project IF/01302/2013 (acknowledging FCT, as well as POPH and FSE – European Social Fund). Côrte-Real, L. thanks FCT for her Ph.D. Grant (SFRH/BD/100515/2014).

**MP143 Uptake and sublethal toxicity of nanogold (nAu) in the nematode *Caenorhabditis elegans***

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The nematode *Caenorhabditis elegans* is used as an ecotoxicological model species in both aqueous medium and in solid substrates. It is easy and low-cost to maintain in the laboratory and it produces hundreds of offspring within a short period of time. It also has a small body size (1 mm), making it possible for in vivo assays to be conducted in 12-well plates. Engineered nanomaterials (ENMs) are a class of emerging pollutants. Nanogold (nAu), is used in many consumer products and in vivo drug delivery. These materials can be released in to the aquatic environment during production or discarding of consumer products. As nAu is insoluble in water, the sediment would become the final depository for the materials. It has become increasingly important to use sediment dwelling organisms to screen for possible toxicity of these ENMs. In this study *C. elegans* was exposed to a range of concentrations of nAu and ionic gold in M9-media, acting as a substitute for pore water. After 96 hours growth, fertility and reproduction was determined. Ultrastructure damage and internalisation of particles in *C. elegans* was determined by using TEM and CytoViva hyperspectral imaging. From these images the nanomaterials are distributed within the reproductive organs, as well as the intestine. Results obtained indicate that nAu affects reproduction more than growth, albeit at very high exposure concentrations, indicating no toxicity at environmentally relevant concentrations. These results give more information regarding the toxicity and in vivo uptake of nAu and form part of an environmental risk assessment of ENMs.

**MP144 Parabens and their metabolites in pet food and urine from New York State, USA**

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In recent years, there is a growing concern on pet animals' health as they are exposing to several chemicals in indoor environment, as like humans. Hence, pets can serve as a sentinel to human exposure. In this study, we determined the concentrations of parabens and their metabolites in commercially available cat ($n$ = 35) and dog foods ($n$ = 23) as well as in urine samples (cat, n = 30; and dog, n = 30) collected from the Albany area of New York State, United States. Mean concentrations of total parabens (i.e., sum of parabens and their metabolites) in dog and cat food were 1350 ng/g and 1550 ng/g fresh wt, respectively and in dog and cat urine were 7230 ng/mL and 1040 ng/mL, respectively. The detected concentrations of parabens and their metabolites indry food were higher than that found in wet food. Similarly, cat food contained higher concentrations of target chemicals than the dog food. In both pet food and urine, methyl paraben (among parabens) and 4-hydroxy benzoic acid (among metabolites) were predominantly found. We estimated the daily dietary intake (EDI) and cumulative daily intake (CDI) and found that there are other possible sources for paraben exposures to the dogs, whereas, for cats, diet is the most of exposure source.

**MP145 Urinary concentrations of phthalate metabolites in pet cats and dogs: A comparison and risk assessment study**

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In recent years, there is a serious concern on raising health issues in pet animals due to their wide exposure to environmental chemicals that are present in indoor, food and other consumer products. Phthalates (diesters of phthalic acid) are one such group of chemicals have been widely used in several commercial and consumer products as plasticizers, solvents/additives and flame retardants. However, they are linked to several health outcomes in human due to their endocrine disrupting activities. To the best of our knowledge, studies on pet animal's exposure to phthalates are scarce. Hence in the present study, we determined the concentrations of 22 metabolites of phthalates (PhMs) in pet cats (n = 50) and dogs (n = 50) urine collected from New York State, USA. The total PhMs concentration in cats was ranged between 64 to 6500 ng/mL, whereas in dogs it was 13 to 3300 ng/mL. The median concentration of total PhMs was ~5 folds higher in cats (600 ng/mL) than in dogs (128 ng/mL). Overall, 9 PhMs were consistently found in all the studied cats and dogs urine (*df %* 100). PA (non-specific metabolite) was the highest abundant chemical in both cats and dogs. We did not find any significant difference in concentration profiles between age groups and sex ($p > 0.05$) among the studied set of samples. Based on the estimated daily intakes (EDI) and Hazardous quotient (HQ) values, cats were at higher risk than dogs due to the expose to phthalates.

**MP146 Acute Toxicity of Corexit 9500A and Assessment of Dioctyl Sulfosuccinate for Monitoring Four Oil Spill Dispersants Applied to Diluted Bitumen**

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Oil dispersants are complex mixtures of solvents and surfactants used to reduce the surface tension of oil slicks through amphipathic surfactant interactions. By encouraging oil droplets to move into the water column from the surface, dispersants minimize surface water contamination and aim to reduce the impact of a spill on coastal wildlife. Future diluted bitumen (dilbit) transportation projects in British Columbia are forecasted to increase coastal tanker traffic, making it important to consider effective oil spill remediation techniques. This study investigated the acute toxicity of the oil dispersant Corexit EC9500A in fish species relevant to the British Columbia coast and assessed the suitability of the commonly used oil dispersant indicator, dioctyl sulfosuccinate (DOSS), in the presence and absence of dilbit. Corexit EC9500A 96-h median lethal concentration (LC50) bioassays were conducted for the freshwater species rainbow trout and seawater species coho, chinook, and chum following standard Environment and Climate Change Canada reference testing method. The final LC50 values for tested seawater species ranged between 35.3 and 59.8 mg/L. Variability in DOSS recovery throughout LC50 testing prompted an investigation of DOSS as an indicator for four DOSS containing oil dispersants Corexit EC9500A, Finasol OSR52, Slickgone NS, and Slickgone EW. For up to 13 days at 5°C and 8 d at 10°C, the DOSS indicator was found to be stable. At temperatures ≥15°C, however, the DOSS indicator was found to be stable for only 3 d, after which DOSS recovery became variable and dependent on environmental factors such as temperature, microbial activity, and aeration. From this study, it was concluded that DOSS is a discrepant indicator and may not accurately correspond to other dispersant components after its application to seawater oil spills.

**MP147 Contaminant mixtures and predicted effects in wadeable streams of the southeastern United**

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Complex contaminant mixtures and associated aquatic-health effects are growing concerns. Replicate (n = 3-10) water samples were collected from 54 small Piedmont (USA) streams over 10 weeks in 2014 and analyzed for 489 organic analytes (470 unique chemicals), including 110 pharmaceuticals and degradates, 226 pesticides and degradates, 84 volatile organic chemicals, and 69 organic waste indicator compounds. Of these, 264 (56%) were detected at least once across all sites. Approximately 70% of the detected water contaminants were designed-bioactive organic chemicals (e.g., pharmaceuticals, pesticides). Due to multiple modes of action, high bioactivity, biorecalcitrance, and direct environment application (pesticides), frequent detection of designed-bioactive organics (range up to more than 100; median greater than 40 per site) at cumulative concentrations up to $\mu g\ L^{-1}$ raise concerns for sub-lethal effects to sensitive aquatic species and lifecycle stages. The possible effects of the complex chemical exposures observed in this study were computationally predicted using knowledgebase-leveraging tools.

**MP148 Determination of the Biosorption Capacity and Biochemical Response of *Chlorella vulgaris* Upon Exposure to Elevated Manganese Concentrations**

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Manganese is introduced into water sources by mine runoff, fracking, and industrial waste processes. Overexposure to Mn leads to a variety of health issues in humans, including progressive, irreversible neurodegenerative effects, increased heart rate and blood pressure, liver damage, decreased fertility, and increased fetal abnormalities. A possible remedy of this problem may be found in algae, which has been used to remove contaminants such as phosphates and nitrates from water sources in previous research. Ideally, the algae could then be harvested to produce value added products, such as triaclyglycerols for use in biodiesel production. *Chlorella vulgaris* is a unicellular green microalga that can produce up to 41 % lipids per dry mass with a short doubling rate, making it an ideal biofuel feedstock. While it has the ability absorb heavy metals, high concentrations may prove toxic, reducing the bioaccumulation ability. In studies of Cd, Cu, and Zn, *C. vulgaris* experienced metal toxicity with symptoms including reduced cell growth, decreased pigment concentrations, and decreased production of proteins and carbohydrates. The objective of this study was to determine to what extent and the mechanism for how Mn bioaccumulates in *C. vulgaris* and how increased Mn concentrations affect the biochemical composition of the alga. This information could be used to determine if the alga is a viable organism to use for bioremediation. Furthermore, if the biochemical processes are not negatively affected, it could be harvested to produce biofuels after cleaning up the polluted waters. The goal of this experiment was accomplished by growing the algae in medium with increased Mn concentrations, assaying for the changes in metabolic processes, and then determining the intracellular and extracellular Mn concentrations via inductively coupled plasma optical emission spectroscopy. The cultures were also monitored for lipid synthesis efficiency, pigment accumulation, photosynthetic efficiency, carbohydrate production, and cell mass. Preliminary results indicate that *C. vulgaris* has the capacity to bioaccumulate up to 1.25 mg/mL of Mn while showing a maximum of a 25% reduction in terminal turbidity. Additionally, the algae grown in increased Mn concentrations take longer to reach maximum photosynthetic efficiency, but maintain the peak photosynthetic efficiency for a greater amount of time. As a result, we expect the cells will produce more carbohydrates and lipids.

**MP149 Temporal and Spatial Trends of Organochlorine Compounds in Herring Gull (*Larus argentatus*) Eggs from the Great Lakes in Michigan, USA, 2002-2017**

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The Clean Michigan Initiative-Clean Water Fund has supported a 15-year-long biomonitoring program of several organochlorine (OC) contaminants in herring gull (*Larus argentatus*) eggs from Lake Erie, Lake Huron, Lake Michigan, Lake Superior, and the St. Mary's River in Michigan from 2002-2006 and 2008-2017. This monitoring study provides a dataset from the United States that mirrors the Environment and Climate Change Canada's (ECCC) Great Lakes Herring Gull Contaminant Monitoring Program dataset. These contaminants include polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and several organochlorine pesticides such as dichlorodiphenyltrichloroethane (DDT) and its ubiquitous metabolite dichlorodiphenyldichloroethylene (DDE). 77 compounds in total were analyzed, as well as 60 TEQs calculated for individual PCBs, PCDDs, and PCDFs. Every year, thirteen eggs were randomly selected from each colony (two colonies per Lake/River, one egg per nest) and analyzed via gas chromatography (GC) at the Great Lakes Institute for Environmental Research, using a similar protocol used by ECCC. Eggs were either analyzed individually or as composites and spatial and temporal trends were assessed for burdens in eggs. Additionally, a Wildlife Contamination Index (WCI) was calculated using several legacy compounds including organochlorines, PCDDs, and PCDFs to assess risk to piscivorous wildlife, rank colonies based on exceedances of guidelines and assess temporal changes in exceedances of guidelines at individual colonies. Calculation of WCIs is ongoing. It is hypothesized that this metric will correlate to either TEQs for each class of compound or the chemical concentrations in the eggs as assessed by GC. General trends in concentrations over this period of time reveal that Lake Erie is ranked the most contaminated for ΣPCBs, octachlorostyrene, dieldrin, *α*-Chlordane, *p,p'*-DDT, and *p,p'*-DDD. Lake Superior is ranked the most contaminated for *β*-Hexachlorocyclohexane, *oxy*-Chlordane, and *cis*-Nonachlor. These and other values in some cases exceed the NOAELs or LOAELs for double-crested cormorants (*Phalacrocorax auritus*), which stand in for toxicity reference values for herring gulls. These data taken together demonstrate that the Great Lakes still carry a relatively high burden of legacy contaminants that may be affecting the health of avian populations.

**MP150 Site-Specific Bioaccumulation Study of Dioxin/Furan Uptake at a Wetland: Consequences to Decision-Making**

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Polychlorinated dibenzo-dioxins/furans (PCDDs/PCDFs) are substances of great concern because of their persistence, ability to accumulate in organisms, and high toxicity. PCDDs/PCDFs are a co-contaminant of wood treatment processes and were historically released into an adjacent freshwater seasonal wetland. In addition to providing economic and social benefits, wetlands are among the most productive ecosystems and frequently provide important habitat and food sources for many resident, migratory, and protected wildlife. A bioaccumulation study was conducted to assess site-specific foodchain exposures to PCDDs/PCDFs by wildlife and re-galvanize a stalled Feasibility Study process. In support of scoping a remedy, site-specific uptake rates were then used to derive risk-based target levels that are protective of the site's wetland. A statistically defensible sampling design was negotiated and implemented – data were deemed of sufficient quality to derive site-specific uptake rates. As hypothesized, this study suggested that uptake into wetland invertebrates were well below values published in the scientific literature. A sensitivity analysis was conducted and demonstrated that moisture content of sediments and invertebrate tissue that are typically accounted for through ingestion or uptake rates can have a notable influence on risk-based cleanup targets. Consistent with the overall aim to avoid 'destroying the wetland in order to protect it', these defensible low uptake rates resulted in risk-based cleanup targets that were protective and yet supported limited intrusive and potentially destructive remediation efforts heretofore considered for the seasonal wetland. Comparable cleanup targets were verified by the oversight regulatory agency. Benefits included: [i] a streamlined agreement on the scope and proposed remedy options and [ii] protective, cost-effective remedial actions.

**MP151 ChemTHEATRE, a platform to browse the published or public data of environmental monitoring and its use for risk assessment**

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There is a general trend toward the growing importance of open data worldwide. It appears to be essential that development of scientific data repositories be accelerated. In the field of environmental chemistry and ecotoxicology, a huge number of monitoring data on chemicals in various environmental and biological specimens have been reported in scientific journals. However, comprehensive, public repositories to store such valuable data set of the chemicals do not exist; researchers are forced to spend lots of time and cost in collecting and utilizing the published data, when modelling environmental behavior and fate of, and performing the risk assessment for, the chemicals of interest. Therefore, it is desirable that various stakeholders in the field should work together to improve and promote secondary use of the data. To this end, we have created a platform to register and visualize the monitoring data of environmental contaminants, named 'ChemTHEATRE' (Chemicals in the THEATRE: Tractable and Heuristic E-Archive for Traceability and Responsible-care Engagement). To date, data described in more than 66 projects have been registered on the platform. Users can find e-archived chemical concentration data in the environmental and biological specimens each with associated metadata such as sampling date and location, species, and biometrics, in addition to the detailed description of experimental methods. Bridging ChemTHEATRE to AIST-MeRAM (Multi-purpose Ecological Risk Assessment and Management Tool) storing chemical property and/or hazard/toxicity information provides us high accurate and transparent assessment of ecological risk of chemicals. Much effort is currently being devoted to visualizing e-archived data sets, and enhancing available data-model interfaces to simulate global dynamics of chemical pollution, with Finely-Advanced Transboundary Environmental model (FATE), and to promote a series of integrated exposure and effects analyses. It is thus expected that ChemTHEATRE will be not only a dedicated follow-up and forecasting tool of international regulations on pollution control in the light of traceability and responsible-care engagement of chemicals, but also a 'communication theatre' where a variety of stakeholders can improve their risk literacies and develop new projects through open data access.

**MP152 Elucidating the Effects of Arsenic and Estrogen on Wound Healing**

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Arsenic is a naturally occurring environmental contaminant that is harmful to humans at elevated concentrations. Increased levels of arsenic in the environment occur as a result of human activities (anthropogenic) and from natural geologically sourced leaching into ground and surface water. These sources pose an exposure risk above the United States Environmental Protection Agency (USEPA) standard (10ppb) to individuals whose food and water sources become contaminated. Arsenic exposure negatively impacts organ function and increases the risk for developing pathologies and cancer. Some of these effects of arsenic on various pathologies and cancer translate to potentially impacting normal cell function in processes such as wound healing. The current study utilized various in vitro techniques to elucidate arsenic's impacts on wound healing processes such as migration, proliferation, inflammation, steroid signaling, and tissue remodeling in cultured human neonatal dermal fibroblast (skin) cells (hDFn). Additionally, estrogen (17-beta estradiol) was used to potentially reverse the negative effects of arsenic on cellular processes. Estrogen has been shown to interact with arsenic and independently communicate with hDFn cells to help facilitate tissue repair during the wound healing process. Results demonstrated that hDFn cells exposed to concentrations of sodium arsenite ranging from 7.5ppb to 750ppb (environmentally relevant doses) decreased cellular proliferation, migration and metabolic activity compared to untreated controls. However, treatment with estrogen resulted in a mitigation of the negative effects of sodium arsenite, suggesting that estrogen may be interacting with arsenic while promoting in vitro cellular migration.

**MP153 Assessment of exposure to fluoride among young children and adults in low- and high- naturally fluoridated areas of Nigeria**

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Although topical fluoride (F) has proven to reduce dental caries, excessive systemic F ingestion leads to the development of dental fluorosis. Given the narrow gap between optimal and toxic F intake/exposure, considerable interest has been focused on intake, bioavailability, and pharmacokinetics of F. This study aimed to measure total daily fluoride intake (TDFI) from the diet and toothpaste ingestion in two age groups (4-5 and ≥20 years) living in low- and high- fluoridated areas (LFA and HFA, respectively) of Nigeria. Subsidiary aims were to measure F in 24-hour urine, plasma, saliva, hair and nails (fingernail and toenail). Parents were asked to complete a Food Frequency Questionnaire (FFQ) for themselves and their children, the F content of their diet was analysed to estimate the daily dietary fluoride intake (TDDFI), and toothpaste ingestion was estimated through a fluoride exposure questionnaire (FEQ). TDFI was calculated from TDDFI and toothpaste ingestion. Fluoride concentration in plasma and nails was measured by fluoride ion selective electrode (F-ISE) after overnight HMDS-facilitated diffusion. Urinary F excretion was determined after direct analysis by F-ISE. TDFI [mean(SD)] for children and adults respectively was 0.075(0.036) and 0.036(0.020) mg/kgbw/d in LFA (F concentration in drinking water ($F_W$) =0.04 mg/L) and 0.277(0.184) and 0.125(0.093) mg/kgbw/d in HFA ($F_W$=3.05 mg/L). In the HFA, TDFI was higher than the threshold of the tolerable upper intake level (UL: 0.1 mg/kgbw/d) for 80% of the children and 50% of adults, whereas in the LFA, 12.5% of the children exceeded the UL. There was a statistically significant difference ($p< 0.001$) between HFA and LFA for all the biological markers among both children and adults. Fluoride intake in the present study was mostly from the diet. Despite a low $F_W$ in the LFA, few of the children are at risk of dental fluorosis. However, most of the children living in the HFA are at risk to dental fluorosis. Therefore, the fluoride level of their biomarker samples will be useful in identifying children at risk of dental fluorosis.

**MP154 Nickel concentrations in blood from informal workers exposed and possible health effects in Colombian**

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In recent decades, the widespread use of heavy metals in modern life to improve our quality of life has caused many of these to come into contact with the environment either from natural sources or as a result of anthropogenic activities, causing various health problems, also to adverse health effects related to oxidative stress, DNA damage and possible carcinogenic; derived from its toxicity, non-biodegradability, bioaccumulation, biomagnification and persistence in the environment. In Colombia, many people earn their daily living doing informal work activities, this includes all those people who are engaged in small economic activities that take place outside the law; Therefore, in most cases, they do not receive any help in terms of professional training in environmental health, nor are they regulated by entities of public health, although in Colombia there is strict regulation on health in the workplace. The development of informal work activities without protection when cutting, welding, using, manipulating and melting some metals, is a direct exposure to Nickel (Ni), derived in activities of: welding, mechanics, carpentry, electricity, smelters, battery repair, electroplating among others. Nickel has not been recognized as an essential element in humans so it is not clear how nickel compounds are metabolized. For each individual the concentration of Ni in blood was determined by graphite furnace atomic absorption spectroscopy (GF-AAS), in a Thermo Fisher Scientic iCE Series 3500. The concentrations of Ni found in blood vary in values from non-detectable

(ND) to 14.3 µg/L Ni total. The average concentrations ± standard deviation (range) were in battery repairers: 4.5 ± 2.2 (1.78-7.91), fishermen: 4.2 ± 1.32 (1.8-9.76), electricians: 4.8 ± 1.5 (2.9-7.0), mechanics: 4.8 ± 2.1 (1.9-14.3), painter 5.1 ± 0.7 (4.2 -11, 7), welding: 3.8 ± 1.2 (1.9-8.0) and recyclers: 1.7 ± 1.6 (0-5.1). For to protect workers, OSHA has set an enforceable limit of 1.0 mg nickel/$m^3$ in workroom air to protect workers during an 8-hour shift over a 40-hour work week. We find the lack of reference values for nickel in blood, both at the general level and in the workplace, therefore, given the shortage of available data, the results that we contribute in our study they can be an important contribution. Acknowledgments: To Colciencias and the University of Cartagena for financing the project No. 110765843679.

### MP155 Dietary intake of trace elements in Catalonia (Spain): Temporal trends and health risks

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Metals are natural components of the Earth's crust which can be widely present in different environmental matrices such as soil, water and air. These elements are environmentally persistent. Consequently, they can bioaccumulate in living organisms causing severe health effects. Therefore, human health risk assessments are required in order to protect human health. This study was aimed at estimating the current dietary intake of arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg), inorganic arsenic (InAs) and methylmercury (MeHg) through a total diet study (TDS). Temporal trends with respect to previous surveys performed in 2012, 2008, 2005 and 2000 were also determined. Food samples were purchased in 12 different cities of Catalonia. Food was selected among those most consumed according to ENALIA survey. For metal analyses, three composites were prepared for each food item, which in turnconsisted of 20 individual units. Regarding to human health risk, a probabilistic approach using Monte Carlo simulation was carried out. Dietary intake estimated for a male adult was 98.16, 2.58, 6.13, 6.38, 5.20 and 2.62 µg/day for As, InAs, Cd, Hg, MeHg and Pb, respectively. These values are below tolerable daily intake (TDI) set by international organisations. In addition, an important decrease compared with previous studies was noted for all elements. Notwithstanding, children population are at risk for cadmium and methylmercury because dietary intake values are above established security levels.

### MP157 Exposure assessment of equol and its chlorinated by-products

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This study simultaneously determined the concentration of equol and its chlorinated by-products in 1013 urine samples collected from 16 major cities across China using a sensitive dansylation LC-MS/MS method. Equol was widely detected in the urine samples with mean concentration of 33.7 ng/L (ND-312 ng/L), and chlorinated equols were for the first time detected in urine samples with concentrations of 8.0 ng/L (ND-1147 ng/L) for monochloro-equol and 0.39 ng/L (ND-144 ng/L) for dichloro-equol. The detection frequency in urine samples was 96% (970/1013) for equol, 21% (211/1013) for monochloro-equol, and 11% (111/1013) for dichloro-equol. The total concentrations of chlorinated equol in urine samples were significantly correlated with that of equol in urine samples ($p$=0.00005), suggesting they would be have common source. For well understanding the potential source, we further analyzed their occurrence in drinking water in China. The concentrations of equol and its chlorinated by-products in drinking water of 58 DWTPs across China were determined, we observed a lower level of equol in drinking water than that in urine samples (10.7 ng/L), and the mean concentrations of monochloro-equol (8.94 ng/L, ND-237 ng/L) and dichloro-equol (0.52 ng/L, ND-12.1 ng/L) in drinking water were similar to those in urine samples.

### MP158 Changes in Concentration of Per- and Polyfluorinated Substances in California Women's Serum

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Per- and polyfluorinated substances (PFASs) have been used in diverse industries from cosmetics and textiles to aerospace and automotive for several decades. Because of their chemical structure, PFASs are highly resistant to degradation and therefore bioaccumulate in food chains. In humans, PFASs bind to proteins in plasma and liver contributing to health problems such as cancer, ulcerative colitis, thyroid disease, high cholesterol, and pregnancy induced hypertension. Many studies have focused on maternal women's serum as pregnancy has a particular set of developmental and health risks associated with fetal development. Here, we will present trending PFAS data from both women and pregnant women's serum. Serum samples were measured for six perfluorinated carboxylic acids (PFCAs), three perfluorosulfonic acids (PFSAs), two alkylperfluorooctanesulfonamidoacetic acids (PFOSAAs), and one perfluorooctanesulfonamide (PFOSA) in human serum using an online SPE (Symbiosis Pro, Spark Holland)-HPLC-MS/MS (SCIEX QTRAP 4000 MS/MS) via a multiple reaction monitoring (MRM) method. Extending our previous work which showed downward trends in PFAS concentrations over time (1960s-2010), we report that most of the classic PFASs slowly but continuously decrease to present (2015-2016), likely due to phase-outs of long-chain perfluorochemicals and their precursors (polyfluoro) which occurred in 2010. This trend is consistent with NHANES data for the US general population since 1999/2000. However, we observed that detection frequencies of longer chain PFASs have increased over the years. The views expressed herein are those of the authors and do not necessarily reflect those of the California Department of Toxic Substances Control.

### MP159 Temporal trend of polybrominated diphenyl ethers (PBDEs) in Northern California Pregnant Women

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Polybrominated diphenyl ethers (PBDEs) were widely used as flame retardant. PBDEs have been found in a large variety of products including children's pajamas, furniture, building materials, and foam furniture. Technical mixtures pentaBDE and octaBDE were phased out in 2004, while decaBDE was voluntarily discontinued being used in 2013. PBDEs are a public health concern because they can disrupt the hormone system, especially of estrogen and thyroid hormones, and developmental exposures can increase risk of a number of adverse health outcomes, including effects on child brain development. Thus, previous studies focused on how phase-out of these technical mixtures has affected PBDE levels found in second trimester pregnant women and their developing fetus(s). Our objective in this study is to continue to follow up the temporal trend of major PBDEs since 2014. In addition, we investigate the PBDE distribution among fetal liver, placenta, and maternal blood serum. The serum samples were prepared via solid phase extraction utilizing RapidTrace® Solid Phase Extraction (SPE) Workstation, while the liver and placenta samples were analyzed for PBDEs using manual liquid-liquid extraction. Placenta samples were also freeze-dried prior to manual extraction. All extracts were quantitated using isotope dilution on a gas chromatograph coupled with high resolution mass spectrometer. Our earlier studies showed a significant drop in major PBDEs (sum of BDE-28, -47, -99, -100, and -153) between 2008-9 and 2011-12 (~ 40%), and then plateaued between the 2011-12 and 2014. Contrary to our earlier findings, the more recent study shows the levels of BDE-47 and BDE-99 slightly rising between 2014 and 2015 serum measurements and then continuing to show a downward trend in serum concentrations in 2016. The reason(s) for this episodic upward trend are being investigated, including studying the PBDE distribution in other matrices. Findings from this study will be added to collective biomonitoring data and help track the long-term behavior of these pollutants in the human body and environment. The views expressed herein are those of the authors and do not necessarily reflect those of the California Department of Toxic Substances Control.

**MP160 Critical Review and Update of Relative Potency Values for Selected Carcinogenic PAHs**

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In Canada and other countries, a relative potency approach has been widely used to estimate risks of polycyclic aromatic hydrocarbon (PAH)-containing mixtures in human health risk assessments. Generally, relative potency is defined as the ratio of the potency of the target PAH to the potency of the comparator chemical, generally benzo[a]pyrene (BaP). In 2010, the USEPA's IRIS Program released a draft update of their relative potency approach for PAH mixtures. The USEPA's Science Advisory Board (SAB) (2011a) recommended significant changes to any future update (USEPA, 2011b). Health Canada (HC) decided to explore updating their potency equivalent factors (PEFs) for 8 carcinogenic PAHs guided by the SAB advice for selecting studies and data based on *a priori* criteria. Methods include (1) updated literature search to select studies meeting *a priori* criteria (e.g., environmentally relevant exposure routes such as oral, dermal, etc.), (2) review of selected studies and extraction of data based on additional *a priori* criteria (e.g., studies with multiple doses of BaP and target PAH relative to tumor responses; reliable dose response data), (3) updated benchmark dose (BMD) modeling in support of the development of PEFs, (4) additional analyses due to limited available data meeting the *a priori* criteria, and (5) calculation of the PEFs based only on cancer bioassay data. None of the chemicals had a sufficient database for calculation of PEFs according to our *a priori* criteria, since no PAH had multiple PEFs from multiple studies, although one study met the criteria of multiple doses for BaP and the target PAH. The only study that was appropriate for PEF calculation was Habs et al. (1980), which evaluated several of the target PAHs of interest. This study was given a medium confidence rating relative to the rest of the database, but it may be considered low confidence in absolute terms, due to incomplete reporting and the potential for confounding from age-related tumours. Our analysis indicates that data appropriate for calculating PEFs for several carcinogenic PAHs are based almost exclusively on dermal studies, even though PEFs are most often used for oral exposure. Furthermore, the relevant data are typically based on old testing methods and lack needed dose-response data. New high quality studies that meet the SAB *a priori* criteria and with good dose response data are needed to evaluate whether the potency approach may be valid for carcinogenic PAHs.

**MP161 Evaluating Prop65 Exposure to Ethylene Oxide and 1,4-Dioxane in Personal Care Products**

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Ethylene oxide and 1,4-dioxane have been found in consumer products as impurities in raw materials such as polyethylene glycol (PEG) and PEG derivatives. PEGs are created under a process known as ethoxylation, which can lead to residual ethylene oxide and generation of 1,4-dioxane. Ethylene oxide and 1,4-dioxane impurities are a concern in personal care products as they are recognized by several authoritative bodies as carcinogens, including California's Office of Environmental Health Hazard Assessment, which oversees implementation of the Safe Drinking Water and Toxic Enforcement Act of 1986 (also known as Prop65). Published literature suggests that ethylene oxide and 1,4-dioxane may be present in over 20% of consumer products such as shampoos, sunscreen, makeup, and moisturizers. Under Prop65, the safe harbor exposure limits for ethylene oxide and 1,4-dioxane are the no significant risk levels (NSRLs) of 2 and 30 micrograms per day, respectively. In order to evaluate Prop65 compliance, we have evaluated several types of PEG raw materials and analyzed samples of personal consumer products for ethylene oxide and 1,4-dioxane content. Our findings indicate that ethylene oxide is not present in the personal care products evaluated at detectable concentrations and 1,4-dioxane is detected in less than 6 percent of samples analyzed at concentrations below Prop65 exposure limits requiring warning notification. In general, purity standards for raw materials considered food additives are low enough to reduce exposure to impurities below Prop65 NSRLs.

**MP162 Cleaning product ingredient safety – How have ingredient listings for cleaning products changed over the past 5 years?**

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The present study seeks to compare ingredient listings between two separate surveys of United States cleaning products and their formulations conducted over a five year period as part of the American Cleaning Institute's Cleaning Product Ingredient Safety Initiative (CPISI). CPISI was conducted with the goal of providing hazard data, exposure estimates, and a screening level risk assessment for each ingredient used in member company household cleaning products. To do this, a survey was conducted to capture dish care, multi-purpose, and laundry care products and their ingredient listings. In 2012 a survey of products and their ingredients was conducted that encompassed 1,100 products and resulted in a database of over 13,000 ingredients listings. The survey captured product type and ingredient name for every listing, and captured CAS registry number, function, and concentration where these were available. The 2012 ingredient listings included over 1,200 different ingredient names, some of which were generalized naming conventions, and some of which were synonymous. Listed names were consolidated to produce the Ingredient Inventory, a list of 588 unique name-CAS registry number combinations. Each unique ingredient was assigned a chemical category and functional class. ACI is currently updating CPISI's online databases. This required repeating the survey, which was performed in 2 parts. A re-survey of products was conducted in summer 2016, with a supplemental effort in fall 2017 to capture formulations for new products entering the market in the interim. The resurvey captured data for 1,470 products, producing a database of 17,510 ingredient listings. These listings included 1,842 different ingredient names which have been consolidated to approximately 800 unique name-CAS registry number combinations. Listings and inventories were compared between the 2012 and 2016/2017 surveys and several trends were identified. Ingredient listings in 2016/2017 displayed a higher degree of specificity in naming, with more ingredients having standardized names or CAS registry numbers than in 2012. The findings of this study bear relevance to risk assessment efforts because greater specificity in ingredient listings allows more certainty when searching for and applying hazard data and allows more precision when measuring exposures for a specific ingredient across product uses.

**MP163 Study on a Prediction Model for Daily Concentration Change of Hydrogen Peroxide in Rivers**

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Hydrogen peroxide is used in many ways mainly as bleaching agent for both home and business use. It is now categorized as "Priority

Assessment Chemical Substances" in Chemical Substances Control Law in Japan, which has ecotoxicity with concern for a considerable amount of it remaining in the environment. Although Japanese government will conduct the risk assessment of hydrogen peroxide, it is hard to do because of its dynamically changeable concentration due to high reactivity with other organic and inorganic matter. In addition, hydrogen peroxide could also be produced through solar photochemical processes in both the atmosphere and surface water. In this study, we were developing a simulation model for daily concentration change of hydrogen peroxide concentration in rivers. Since hydrogen peroxide was produced through photochemical processes of dissolved organic matter (DOM) and oxygen, we were also developing a method for simulating concentrations of organic matter in river water. The model was developed based on an existing multimedia environmental fate model, G-CIEMS, and constructed geographic information system data of sewage collection areas and related effluent points of sewage treatment plants. We selected biochemical oxygen demand (BOD) and chemical oxygen demand (COD) as DOM indicators, simulated these concentrations in four river systems (Ashida River, Ohta River, Numata River, and Kurose River) in Hiroshima Prefecture, and compared these simulated concentrations with measured ones which were performed by the Japanese government. Then we simulated daily concentration changes of hydrogen peroxide in river water and compared these with measured concentrations which were collected at Rengeji Bridge, located upper stream of Kurose River, every two hours from August 24 to August 25, 2017. We also simulated influence of direct emission of hydrogen peroxide to river water from home and business use and direct depositions of hydrogen peroxide from the atmosphere which were calculated by an air pollution forecasting system, VENUS, developed in National Institute for Environmental Studies, Japan.

## Approaches and Challenges in Sediment Toxicity Testing for Environmental Risk Assessment

**MP164 Application of an undisturbed sampling technique for depth related analysis of sediment particles and pore-water in OECD 219 sediment test systems**

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Sediment toxicity testing of plant protection products (PPP) has gained an increasing awareness within the scientific and regulatory communities. Currently, PPP concentrations in sediment and pore-water of toxicity tests according to OECD Test Guidelines 218/219 are determined as the mean over the entire sediment layer of the test system. Hence, a depth-related measurement would contribute to a more accurate assessment of the effective exposure for the test organism, *Chironomus riparius*, a predominately surface dwelling organism. Therefore, we developed an undisturbed sampling and processing technique that enables depth-related analysis of active substances in pore-water and substance bound to sediment particles. The test system was designed according to OECD Test Guideline 219. After removal of the water phase, three plastic tubes were inserted into the approximately 15 mm thick sediment layer, which was subsequently flash-frozen with liquid nitrogen. Using a specially developed cutting device, the sediment cores were cut into three slices, with an approximate thickness of 5 mm each. Each sediment slice was centrifuged to isolate the pore-water. A sequential extraction was performed to extract the sediment adsorbed residues. The pore-water and sediment extracts were analyzed by LC-MS/MS. To validate this newly devloped sampling technique, a *C. riparius* toxicity test was conducted according to the OECD 219 spiked-water sediment test method. Two model compounds, A ($logP_{ow} < 1$), and B ($logP_{ow} > 3$) were used as a mixture at nominal concentrations of 2 µg/L overlying water. To investigate the spatiotemporal behavior of the compounds, test systems were incubated and processed 3, 7, 14, 21, and 28 days after treatment. The concentrations of the applied compounds decreased in the overlying water throughout the duration of the study. Both compounds were primarily found adsorbed to the sediment phase (approximately 40 – 50%). Approximately 0.2 – 1% of the applied compounds were recovered in the pore-water at the same time. The analytical results of pore-water and sediment extracts show that the highest amounts of both compounds were in the uppermost layer of the sediment. These first results indicate that the newly developed sampling technique can provide a substantial contribution to a more realistic determination of exposure concentrations in chonic water-sediment toxicity tests, leading to an improved sediment risk assessment.

**MP165 Challenges Experienced During Development of a 96-hour Water Only Toxicity Test with the Midge, *Chironomus dilutus***

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In 2016, a 96-hr water only exposure toxicity test method with *Chironomus dilutus* became required for some stormwater and ambient water monitoring projects in California. As a result, Pacific EcoRisk was asked to propose a method based on the EPA acute testing manual (EPA-821-R-02-012) using 7-day old organisms in order to be consistent with forthcoming revisions to the EPA and ASTM sediment testing manuals.Although "*Chironomus spp.*" is listed in Appendix B of the acute manual on a list of supplemental acute toxicity test species, only test temperature and life stage are provided for test performance. Also, a recent 96-hr *Hyalella* inter-laboratory comparison study using a similar water exposure, identified considerable variability among labs performing tests under different conditions. While our laboratory has considerable experience performing sediment testing with *C. dilutus* (e.g., 10-day to full life cycle), the sediment tests typically have at least a 14-day holding time which allows the lab to obtain egg cases and hatch organisms under controlled laboratory conditions prior to testing. Short notice of stormwater testing does not typically allow the lab to obtain egg cases, hatch them, and raise larvae to 7-days old prior to the standard EPA hold time of 36-hrs for water samples from expiring. Since implementation of this test method, we had an unacceptable frequency of invalid tests when using test organisms shipped from suppliers overnight as 6-day old larvae for testing at 7-day old larvae upon arrival. Therefore, we performed several rounds of QC testing comparing larvae reared from lab hatched egg cases, to larvae obtained from suppliers (either used immediately or after holding for 24-hrs to allow for attrition in the culture), as well as using alternate suppliers of larvae. In order to achieve a greater rate of test acceptability, two updates were eventually made to the test method. The first was increasing the hold time for this test from 36 hours to 48 hours; this provided the ability to hold larval cultures from organism suppliers for a day prior test initiation to allow for attrition of larvae that were weak due to the stress of shipping. The second update was to increase the age range of *C. dilutus* used in testing from 7-days to 7-10 days. These adjustments greatly increased the frequency of acceptable tests, especially for short-notice (e.g., stormwater) events when larvae had to be shipped from vendors.

**MP166 *Hexagenia* spp. in Toxicity Testing: Methodology Investigations and Potential Improvements**

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*Hexagenia* spp. is an ecologically important burrowing mayfly that when used sediment toxicity assessments provides an understanding of the potential toxicity to an organism with very different physiology and ecological niche than the standard test species *Chironomus dilutus* and *Hyalella azteca*. *Hexagenia* spp. have been routinely incorporated into Ontario Ministry of the Environment and Climate Change (MOECC) sediment quality assessments in the province of Ontario, Canada for almost 25 years. *Hexagenia* eggs are collected in the field from adults of the co-existing species *H. limbata* and *H. rigida* which, until recently, could not be distinguished from each other during collection. Due to DNA barcoding it is now possible to distinguish between the adults at the time of egg collection and these two species can be cultured separately. Method improvement studies by the MOECC included species-specific assessments of egg hatch out success and time to first hatch with and without storage, and species-specific growth rates and size variability in the culture. The co-efficient of variation around the potassium chloride reference toxicant tests for the MOECC mixed species *Hexagenia* was variable and sometimes quite high and it was unclear how much of this was due to the species ratio in the tests. Ninety-six-hour, water only, acute lethality tests were run with the two species and a variety of different toxicantsincluding potassium chloride, sodium chloride, ammonium chloride and copper sulfate to assess sensitivity and variability in the two species' responses. In addition, previous method improvement studies with mixed culture determined that the interpretation of the growth endpoint may be influenced by the organic content of the sediments in the 21-day MOECC sediment toxicity test method, in which the organisms are not fed. In this study the impact of species, feeding and organic carbon on the survival and growth of *Hexagenia* was assessed with a dilution series of marginally toxic sediment diluted with low TOC potters clay. The results of these method improvement investigations will be discussed.

**MP167 Toxicity of sediments under the influence of the emissary submarine of Santos using the marine amphipod *Parhyale hawaiensis***

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One option for effluent discharge is the ocean because of its high depuration and dilution capacity. In Brazil, the Santos Submarine Outfall is considered one of the main emissaries of the country, reaching a volume of seven thousand liters per second. However, the practice of effluent disposal in the oceans may have negative impacts on aquatic biota. When treatment is not appropriate several contaminants can reach the water and sediments of the disposal area. This work aimed to perform acute toxicity tests with marine amphipod *Parhyale hawaiensis* with sediment samples under the influence of Santos Submarine Outfall. This organism has become a new model for toxicity tests. It has been shown to be a sensitive organism to several contaminants and is easy to be cultivated in laboratory conditions. Samples were collected at four different sites in four sampling campaigns and were evaluated as fresh sediment, dry sediment (45°C), aqueous fraction (elutriate with sediment and water), 1:4 (w/v), and organic extracts (2.5DCM:1MeOH). Liquid samples were tested using 96-wells microplates, and the sediment using 12 wells-microplates containing sediment and salt water in 1:4 (w/v). Neonates (< 7 days old) *P. hawaiensis* were used and placed individually in each well. Exposure conditions were 96h, 24+2°C, 12h/12h light and dark. At the same time that the tests were being performed, sensitivity tests were done using Zn as reference toxicant. Temperature, pH, dissolved oxygen and salinity of each sample were verified. From the 14 samples analyzed, 72% were toxic when fresh sediments were tested, and 78% when dried sediment were evaluated. All aqueous extracts were not toxic and 71% of the organic extracts tested presented toxicity. We also observed a positive correlation of toxicity with extractable organic matter. Therefore, we conclude that the sediments are being adversely affected by outfall discharge and toxicity is associated to organic contaminants. Acknowledgements: FAPESP 2017/24758-5 and FAPESP 2017/22010-9

**MP168 Effects of metal contamination of streams in the Tri-State Mining District: 1Toxicity of metal mixtures to juvenile mussels in 12-week water-only test**

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Field studies have found that the abundance and diversity of freshwater mussels were reduced in metal-contaminated streams that drain the Tri-State Mining District (TSMD) of Missouri, Kansas, and Oklahoma. However, previous 4-week laboratory toxicity studies indicated that juvenile mussels were not among the most sensitive benthic taxa. We recently conducted a series of longer-term (12-week) toxicity tests to ensure that freshwater mussels are protected from injury from toxic metals in TSMD streams. In this study, we conducted water-only tests to evaluate the contributions of metals from stream water to toxic effects on juvenile mussels. A 2016 survey of TSMD streams found that dissolved zinc concentrations in surface water and interstitial water of mussel habitats frequently exceeded 10 ug/L at most sites downstream of mining activities. Lead and cadmium were detected in fewer samples, with these metals occurring at ratios of about 200 (Zn): 2 (Pb): 1 (Cd). We conducted 12-week tests with juvenile fatmucket *(Lampsilis siliquoidea*) to compare toxic effects of zinc exposure with the effects of a zinc-lead-cadmium mixture. Results of these studies indicated that toxic effects on juvenile mussels (reduced biomass) occurred at lower Zn concentrations in the metal-mixture test, with an EC20 of 7.2 ug/L (expressed as Zn) in the three-metal mixture, compared to 21 ug Zn/L for the Zn-only test. These results demonstrate that juvenile fatmuckets are highly sensitive to toxic effects of zinc and other metals at concentrations occurring in mussel habitats of TSMD streams. Ongoing studies in our laboratory will characterize the relative contribution of metals from TSMD sediment and overlying water to toxic effects on juvenile mussels, with the goal of establishing a reliable site-specific threshold for injury to mussel communities of the TSMD caused by metals.

## Wildlife Ecotoxicology Supporting Management Decision Making

**MP169 Assessing Trophic Magnification of Cyclic Methyl Siloxanes in a Terrestrial Food-Web of an Avian Top Predator, the Cooper's Hawk**

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Several types of legacy persistent organic pollutants (POPs), such as PCBs and DDE, and emerging POPs like cyclic volatile methyl siloxanes (cVMS) are released from multiple sources into the ambient environment and are known to negatively impact endocrine and physiological functions within exposed wildlife. Protocols to assess bioaccumulation of these persistent chemicals within terrestrial systems are far less developed compared to aquatic systems. Presently, regulatory agencies in Canada, the USA, and the EU use only bioaccumulation information for fish to assess the bioaccumulation potential of chemicals. However, recent studies have shown that some chemicals that are not bioaccumulative in aquatic food-webs do biomagnify in terrestrial food-webs. To better understand the bioaccumulation behaviour of chemicals in terrestrial food-webs, we aim to produce a food-web model to assess the biomagnification of POPs in an apex avian predator, the Cooper's hawk. Over 100 samples were collected from various trophic levels of the food-web including hawk eggs, songbirds, invertebrates, and berries. All samples were analyzed for a number of contaminants listed as priorities for monitoring by the Chemical Management Plan of the Canadian federal government. Stable isotope analysis of $\delta^{13}C$ and $\delta^{15}N$ signatures of hawks, songbirds, invertebrates, and berries was used to estimate the trophic position of each organism. Censored regression by maximum likelihood estimation was used to assess the relationship between the natural logarithm of each contaminant lipid equivalent concentration and trophic position. Trophic magnification factors (TMFs) were determined for cVMS and legacy POPs for comparison. TMFs of PCBs, PBDEs, and OCPs ranged from 1.20 to 15.66, 3.11 to 7.32, and 0.77 to 7.79, respectively. Indicating that most legacy POPs are biomagnifying in this terrestrial food-web. TMFs of octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5) and dodecamethylcyclohexasiloxane (D6) were estimated at 0.77 (0.12 SE), 1.17 (0.18 SE), 1.29 (0.23 SE), respectively, indicating that cVMS are either diluting or not biomagnifying in this terrestrial system. Overall, terrestrial TMF values for legacy POPs were comparable to or slightly higher than TMF values determined for several aquatic systems; however, the terrestrial TMF values for the cVMS were lower than TMF values reported in aquatic systems.

**MP170 Biomonitoting Studies of Lead and Organochlorine Pesticides in Southern European Raptor Species**

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The most raptor species suffer the consequences of multiple pressures, such as the exposure to toxic persistent pollutants, which has been widely studied in biomonitoring programmes. These results can be used in risk assessment procedures, helping to decision makers to control adverse effects, and not only on these species, but also in the rest of the living organisms, humans included. In Spain and particularly in the southern area breeding species of migrant and non-migrant raptor are usually observed. The objectives of this study were: 1) to evaluate the exposure to lead and organochlorine pesticides in raptors from Andalusia (southern Spain), 2) to estimate potential risks related to contaminant exposures, and 3) to propose reference levels for management decision making. A total of 439 blood samples were analysed from 15 raptor species, such as Spanish Imperial eagle (*Aquila adalberti*), Bonelli's eagle (*Aquila fasciata*), Egyptian vulture (*Neophron percnopterus*), Griffon vulture (*Gyps fulvus*), Cinereous vulture (*Aegypus monachus*), Black kite (*Milvus migrans*), Eurasian sparrowhawk (*Accipiter nisus*), Eurasian buzzard (*Buteo buteo*), Bearded vulture (*Gypaetus barbatus*), Red kite (*Milvus milvus*), Northern Goshawk (*Accipiter gentilis*), etc. Raptors were chronically exposed to lead suffering sublethal effects. About 90% of non-scavenger individuals had blood lead levels below 20 µg/dL (subclinical threshold). On the contrary, about 13% of the population of obligate scavengers would require intervention. These data suggest the need for a more intensive monitoring in order to establish measures to avoid effects on scavenger populations. Griffon vultures, Bonelli's eagles and Goshawks were the most exposed to organochlorines. The most common organochlorine compounds detected were δ-HCH, lindane, endosulfan I, endosulfan sulfate and *p,p'*-DDE. These results showed that the exposure to organochlorine pesticides in Griffon vultures from Andalusia (2003-2006) followed a pattern similar to that described in vultures from Greece (2003-2004) and very different from that of the vultures from India (2005-2007), which suggests the existence of a "Mediterranean pattern of exposure to organochlorine pesticides". A decrease over time in the levels of organochlorine pesticides was observed, however *p,p'*-DDE levels in scavengers should be periodically monitored. Acknowledgements: Seneca Foundation Project MASCA'2014 (19481/PI/14). ERBFacility CA16224 COST-Action.

**MP171 Comparison of sublethal effects of eight environmental chemicals in adult and early-life stages of Japanese quail**

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Toxicity tests for avian species are generally performed on adult birds. We, and others, have proposed that the development of standardized early-life stage (ELS) toxicity tests represents an advance over current testing methodologies in terms of cost, animal use, and biological relevance. In this study we determine the effects of sublethal concentrations of 8 environmental contaminants (benzo[a]pyrene [BaP], lead (II) nitrate [Pb], seleno-L-methionine [SeMe], hexabromocyclododecane [HBCD], ethinylestradiol [EE2], fluoxetine hydrochloride [FXH], trenbolone [TB] and chlorpyrifos [CPF]) on adult and embryonic Japanese quail (JQ). For both adult and ELS studies, treatment groups included a vehicle control (Adults: corn oil, ELS: DMSO), and three concentrations of each test compound, all of which were predicted to result in < 20% mortality based on existing literature. Adult JQ (6-10 weeks of age) were exposed via oral intubation directly into the crop, whereas ELS JQ were exposed via injection into the air cell of fertilized, unincubated eggs. Liver tissues were collected from a sub-set of individuals (n=5-6 per dose group) mid-way through the experiment (Adults: day 4, ELS: day 9) for 'omics and chemical residue analysis. At the end of the experiment (Adults: day 14, ELS: day 16), the remaining individuals were examined for deformities, growth, and health metrics, and tissues were collected for histology, 'omics, and chemical residue analysis. Overall, no treatment-related mortality was observed in adult birds with the exception of Pb, which caused 100% mortality at the highest dose. Two of the 8 test chemicals, BaP and FXH, caused significant mortality in ELS JQ. The high dose of CPF was associated with a significant increase in developmental abnormalities, and a significant decrease in embryo and gallbladder mass in ELS JQ. Embryo mass was also significantly decreased by the highest dose of EE2, and tarsus length was decreased with low and high doses of HBCD. Analysis of the adult toxicity data as well as histological, 'omics, and chemical residue analysis for both life history stages is ongoing. The co-determination of a range of molecular and organismal level end points at two different life stages will help us to evaluate the feasibility of using a standardized ELS toxicity test for the evaluation of environmental chemicals in birds. This study is part of the EcoToxChip project (www.ecotoxchip.ca).

**MP172 Contaminants and cross-ecosystem linkages: food web implications for wildlife**

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Contaminants can impact ecosystems by reducing the quantity and quality of aquatic insect prey available to aquatic and terrestrial consumers. Understanding these impacts allows us to predict hotspots of contaminant flux (i.e., prey production x contaminant concentration) through food webs. We summarize results from two studies examining flux of current-use pesticides and methyl-mercury from freshwaters to terrestrial food webs in adult aquatic insects to illustrate the importance of this approach to support wildlife management decisions. In the Prairie Pothole Region of North America, we found that concentrations of insecticides such as bifenthrin and imidacloprid in adult insect tissues were negatively correlated with production of adult aquatic insects during the bird breeding season. However, pesticide flux was highest from agricultural wetlands because of increased productivity of those systems due in part to nutrient inputs. In the upper Salmon River watershed in Idaho, we estimated that methyl-mercury flux from aquatic to terrestrial food webs downstream of an abandoned mercury mine increased below the point source, despite the fact that concentrations accumulating in the food web decreased, because of larger stream surface area. Adult aquatic insects emerging from these freshwaters are important prey for young waterfowl, aerial insectivorous birds and aquatic predators such as fish, and can act as vectors of contaminant transfer to multiple food webs. Non-intuitive patterns of contaminant flux result from the varying effects of habitat and contaminant on insect production. Landscape models of regional emergence and contaminant flux by adult aquatic insect prey can aid in decision making by terrestrial and aquatic wildlife managers.

**MP173 Estimating Cadmium Risks to Small Mammals: Dietary Doses and Tissue Concentrations**

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We reviewed cadmium (Cd) toxicity data to identify toxicity reference values (TRVs) applicable to small mammal survival and reproduction, with the goal of protecting local populations. The U.S. Environmental Protection Agency (USEPA) identified a mammalian TRV for Cd as 0.77 mg/kg-day, which is a NOAEL for rat growth. Growth is sometimes used as a surrogate for reproductive fitness, but this approach has limited applicability for mammals. Mammals tend to stop growing once adult size is reached, and slower initial growth can often be compensated later. Also, applicability of a rat NOAEL to small mammal TRV derivation is limited due to differences in body size, which affects exposure-effects relationships through differences in metabolism and tissue concentrations. We considered two lines of evidence for TRV development: (1) a literature review of multigenerational studies as a direct measure of Cd effects on reproductive fitness, and (2) published tissue-based TRVs. Multigenerational Cd studies are available for rats, mice, and bank voles, with NOAELs ranging from 0.0069 to 12 mg/kg-day, and LOAELs ranging from 1.5 to 2.7 mg/kg-day. The lowest LOAEL is uncertain as it reflects a behavioral effect (infanticide) without a strong dose-response relationship. Thus, a TRV range of 1.5 to 2.1 mg/kg-day is identified. For the tissue line of evidence, published TRVs have focused on renal dysfunction as the most sensitive effect, which could potentially affect survival or have a secondary effect on reproduction. This endpoint is considered conservative, because it is not a direct measure of survival or reproductive fitness. To model risks based on tissue TRVs, Cd in small mammal tissue was estimated from Cd in diet. Paired diet and tissue data for shrews were used to develop a diet-to-tissue bioaccumulation regression. This approach provides a means to address the difference in body weight and metabolism between rats (basis of TRVs) and shrews (highly-exposed species). Despite the conservative effects endpoint underlying the tissue TRV, this approach yields lower risk estimates for shrews than the dietary dose approach. We suggest that the tissue-based approach better accounts for body weight differences, and we provide a sensitivity analysis for the dose-based approach using body weight scaling as applied by USEPA for human health risk assessments.

**MP174 Influence of non-breeding season movements on patterns and trends of POPs in seabird**

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There is a continuing need to monitor ecosystem contamination by xenobiotic compounds, particularly those, such as POPs, which are persistent and accumulate in food chains. Seabirds have proven to be an efficient and effective means of tracking POPs in the marine environment, and we have previously published data on long term monitoring of seabirds in the Northeast Pacific, mainly using egg samples. Seabird species can, however, be migratory with complex movement patterns outside the breeding season which can affect exposure to contaminants. Here we present data on two representative species, the rhinoceros auklet, *Cerorhinca monocerata*, a feeder mainly on small pelagic fishes, mainly over continental shelf habitat, and the ancient murrelet, *Synthliboramphus antiquus*, which uses similar habitat but feeds on more of an invertebrate diet. Both species have complex movements once they leave the breeding colonies. At a number of those colonies we collected eggs from the auklet and blood samples from the murrelet of individual birds which had been fitted with geolocator devices during the previous breeding season, and then tracked until their return to the colony the following spring. Samples were analyzed for PCBs, organochlorine pesticides, brominated flame retardants, perfluorinated compounds and stable isotopes. Data will be presented on the influence of wintering area on concentrations of those POPs compounds as discerned from telemetry and stable isotope results.

**MP175 Life history and body mass changes can influence contaminant concentrations in animals: Examples with mercury contamination in wild birds and mammals**

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Mercury (Hg) contamination of oceans and inland waters is prevalent worldwide, posing a threat to animals in many ecosystems. Most ecotoxicological studies focus on exposure to humans and animals and the associated toxicological risk. However, the physiological changes that animals undergo throughout their annual life cycles can impact whether contaminant exposure may lead to adverse effects. Specifically, large, and sometimes rapid, changes in an animal's body mass can substantially alter contaminant concentrations. We joined the results from research in a mammalian and avian system to demonstrate how Hg concentrations can be influenced by substantial changes in mass as a result of life history events. We quantified Hg concentrations in northern elephant seals (*Mirounga angustirostris*) before and after lengthy at-sea foraging trips or fasting periods on land and showed that Hg concentrations in blood and muscle changed in response to these events. Adult female elephant seal blood Hg concentrations decreased by 30% across each of the two annual foraging trips, demonstrating a foraging-associated dilution of Hg concentrations as seals gained mass. In contrast, blood Hg concentrations increased by 103% and 24% across the two annual fasting periods (breeding and molting, respectively), demonstrating a fasting-associated concentration of Hg as seals lost mass. For Forster's terns (*Sterna forsteri*), we quantified Hg concentrations in chicks that were repeatedly sampled during the pre-fledging period when birds were rapidly gaining mass. Similarly to elephant seals, blood Hg concentrations decreased as tern chicks increased in body mass. Furthermore, we observed a strong relationship between the proportional change in chick blood Hg concentrations and the proportional change in body mass. In contrast, for seals, the proportional change in blood Hg concentrations was related to the proportional change in body mass while foraging but not fasting. Many small- and large-bodied animals undergo substantial changes in mass as a result of growth, migration, breeding, fasting, hibernation, or other life history events. Consequently, regardless of environmental exposure to Hg, an animal's toxicity risk may also be markedly influenced by changes in body mass.

**MP176 Monitoring reproductive and immunological effects in colonial waterbirds to support management decisions at contaminated Great Lakes sites**

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This monitoring program assessed effects of contaminants, primarily PCBs and PCDDs, on immune function and reproduction in fish-eating birds in the Saginaw Bay and River Raisin Areas of Concern (AOCs) and Grand Traverse Bay during 2010-18 under the Great Lakes Restoration Initiative and AOC programs of the US Fish and Wildlife Service and US Environmental Protection Agency. Saginaw Bay sites included two herring gull colonies (Confined Disposal Facility (CDF) and Little Charity Island), two Caspian tern colonies (CDF and Charity Reef/L. Charity Island) and one black-crowned night heron colony (CDF). Herring gulls were studied in the River Raisin AOC at the Detroit Edison Monroe Power Plant on the western shore of Lake Erie and on Bellow Island in Grand Traverse Bay. Reference sites were in the lower St. Mary's River (gulls on Pipe Island Twins and terns on Two Tree Island), on Taquamenon Island in Whitefish Bay (terns), and on Chantry Island, Lake Huron (herons). Gull embryos were assessed during late incubation using a viability detector sensitive to heartbeat and movement. Relative risk ratios for embryonic nonviability were significantly elevated two to four-fold at contaminated sites compared to the reference site (2.1 for the Saginaw Bay AOC, 2.7 for the River Raisin AOC, and 4.1 for Grand Traverse Bay). Infertility was the primary cause of nonviability at the reference site. Elevated infertility and mortality contributed to nonviability in contaminated sites. Deformities associated with PCBs and PCDDs were found in several individuals at AOCs (3 gull chicks at Monroe, 2 tern chicks on L. Charity, 2 gull embryos on the CDF, and 1 gull embryo on L. Charity). Chick productivity in terns in Saginaw Bay (mean of 0.9 chicks/nest) was significantly below that of reference sites (1.3 chicks/nest). In the River Raisin AOC, productivity of gull chicks was poor in 4 of 8 years, with complete reproductive failure during 2010. In gull chicks the mean phytohemagglutinin (PHA) skin response for T-cell mediated immunity was suppressed 52-55% at both AOCs and 46% in Grand Traverse Bay. This response was suppressed 46% in terns and 39% in herons in Saginaw Bay. Mean antibody responses in gull chicks at the River Raisin AOC and in Grand Traverse Bay were two to three-fold lower than at the reference site. Continuing immunological and reproductive impairments at these contaminated sites are consistent with the effects of persistent pollutants such as PCBs and PCDDs.

**MP177 New Approach to Developing Avian Toxicity Reference Value for Selenium**

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Toxicity reference values (TRVs) are used in ecological risk assessments to assess risk to wildlife species using hazard quotients. TRVs are threshold doses for risk typically developed from laboratory toxicity studies using domestic species easily raised in captivity, and as such the results of laboratory conditions and species responses may not reflect the conditions or actual risk to wildlife in the field. For metalloids such as selenium that bioaccumulate in eggs, other approaches are possible and more realistic. Because the egg is a sensitive stage for selenium toxicity and its concentration is correlated to hatching success, the egg is the ideal stage to develop avian selenium TRVs. Egg concentrations and hatchability can be measured in field nests, removing the constraint of using only laboratory studies to develop avian TRVs. In addition, a dose-response curve provides more comprehensive information for decisions than single threshold doses traditionally used. As such, a new three-step approach was applied to define a dose-based avian TRV for selenium more representative of field conditions for a former phosphate mining site in Southeast Idaho. First, dose-response curves on egg concentrations were fit to data available for 11 bird species with adequate nest datasets (pooled across studies in a meta-analysis) to predict hatching success. One (EC1) and ten (EC10) percent effect concentrations were derived from each curve. Second, to translate the egg concentration threshold to a dose-based threshold, a trophic transfer factor (TTF) of selenium from diet to egg was estimated for each species using available co-located data from field and laboratory studies. This TTF was divided into the egg concentration threshold to predict a no- and low-adverse effect level diet concentration threshold. The third step completed conversion to dose-based thresholds by multiplying the diet concentration threshold by the body-weight normalized ingestion rate for each species. Final dose-based thresholds were calculated for each species and compared to reproductive success reported for the bird community in Southeast Idaho exposed to elevated selenium on former phosphate mines. The geometric mean for the set of species provided a selenium TRV that is more consistent with reproductive success of bird populations in this area than the traditional TRV developed solely from laboratory studies. This more realistic TRV can help managers make more informed remedial decisions.

**MP178 New Approach to Developing Mammal Toxicity Reference Value for Selenium**

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Toxicity reference values (TRVs) are used in ecological risk assessments to assess risk to wildlife species using hazard quotients. TRVs are typically threshold doses for risk developed from laboratory toxicity studies that may or may not be representative of conditions in the field. In particular, laboratory studies determining selenium doses for mammalian toxicity thresholds do not match observations of toxicity in mammals in the field. Therefore, a TRV for selenium was developed based on field data and compared to laboratory-based TRVs. Field data evaluated included mammal studies on reproduction in areas with high selenium concentrations in the soil, sediment, vegetation and invertebrates at the Kesterson Reservoir on the Kesterson National Wildlife Refuge in southern California. Data collected from field studies conducted at Kesterson over 14 years resulted in no correlations of selenium in mammal tissue with reproductive effects. In addition, comparisons to reference areas did not reveal obvious selenium effects on the mammal community that could be differentiated from effects of differences in the habitat conditions. A 10-year biomonitoring dataset collected at Kesterson provided a data distribution for vegetation and invertebrates at Kesterson, which was used to develop a no observable adverse effect level (NOAEL) dose for the representative mammal species at the site. To corroborate this threshold level, a dose-response curve was developed using a meta-analysis approach of combining data from laboratory studies provided in USEPA's Ecological Soil Screening Level (EcoSSL) document for mortality, mortality of juveniles only, and reproductive endpoints for all mammal species in the EcoSSL database. The ten percent effect concentration ($EC_{10}$) of the dose-response curves for most species groupings were similar to the NOAEL estimated using the Kesterson field data.

## Advances in the Toxicological Assessment of PAHs

**MP179 Application of Surface Enhanced Raman Spectroscopy (SERS) for rapid PAH detection in water bodies**

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Analytical methods for chemical detection in water bodies are often costly, time consuming and require special skills. Surface Enhanced Raman Spectroscopy (SERS) is a very attractive, non-invasive optical technique that allows rapid detection of chemicals in low concentrations and does not require large amount of sample (µL range). Authors will discuss and demonstrate the novel liquid SERS technique application we are developing to measure and analyze the 16 EPA priority PAHs. Colloidal liquid gold substrates are applied to enhance the Raman signal of the PAHs down to concentration of 1ppm. SERS spectra have been recorded applying a 785 nm laser with integration time of only 5 to 10s. This technique also allows reducing spectral shift, which can happen due to chemical interaction of analyte and substrate in conventional SERS. It is possible to identify PAHs individually or in mixtures as well as in natural water bodies. We performed real lake water PAH spiking tests to demonstrate applications of the technique. Colloidal liquid gold substrates are remarkably stable and allows us to store samples in the freezer for long times without noticeable degradation of the Raman signals.

**MP180 Assessing the risks from sediments highly contaminated with PAHs in Owen Sound Bay, Canada**

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Sediments in the inner harbour of Owen Sound Bay in Lake Huron, Ontario, Canada are heavily contaminated with polycyclic aromatic hydrocarbons (PAHs). Proposed dredging in the harbour may pose risks to resident fish populations and to the spawning grounds of lake whitefish (*Coregonus clupiformes)*. In this study, we investigated the ecotoxicological risks posed by PAH contamination in the inner harbour of Owen Sound Bay. Through the use of SPMD passive samplers, we showed that the estimated concentrations of PAHs in the water column in the inner harbour are elevated to concentrations that exceed provincial water quality guidelines. Sediment cores were collected from several locations in the inner harbour and from a nearby reference site in Lake Huron. Total PAHs in sediments were detected at concentrations as high as 48 mg/kg dry weight, with no indication of declining concentrations to the bottom of the sediment core (i.e. 25 cm). These PAH concentrations are consistent with the levels of sediment contamination in several Areas of Concern in the lower Great Lakes. In order to make some predictions on whether dredging the sediments in the inner harbour of Owen Sound Bay pose risks to fish, sediment disturbance simulation experiments were completed with juvenile lake whitefish. We observed changes in biomarkers of exposure to PAHs, as indicated by slight induction of EROD activity, as well as significant oxidative stress, as indicated by elevated TBARS activity ($p < 0.05$). Overall, these results provide lines of evidence for assessing the ecological risks associated with PAH contamination in the inner harbour of Owen Sound Bay, and it is hoped that these data will influence future management decisions regarding dredging and/or remediation activities within the harbour.

**MP181 Benzo(a)pyrene Exposure (BaP) induces Gene Expression and Metabolic Pathway Changes in Male Tilapia**

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Benzo (a) pyrene (BaP), the prototype of polycyclic aromatic hydrocarbons, is known to exhibits genotoxic and carcinogenic effects. Cancer promotion of BaP is associated with the activation of apoptotic signals and may induce survival signals. Recently, it has been reported that disruption in metabolic pathways has a role in carcinogenesis. One of the most studied hallmark in cancer is the Warburg effect (aerobic glycolysis). Despite a wide number of toxicological studies that describe BaP effects, the metabolic mechanisms that underlie these effects in fish are largely unknown. Of great concern is the presence of BaP in aquatic systems, especially those in close proximity to human activity leading to consumption of potentially contaminated foods. In this study we focus on the effects of repeated BaP exposure (3 mg/kg) in a short period of time (26 d) on gene alteration and changes to metabolic pathways associated with the Warburg effect in male adult tilapia. Using high-throughput RNA-Seq we identified that BaP exposure induced important changes in the gene expression of genes related with energy metabolism like hexose-6-phosphate dehydrogenase (glucose 1-dehydrogenase), acetoacetyl-CoA synthetase, malate dehydrogenase 1B and succinate dehydrogenase complex in the liver. In addition genes involved in glycolytic activities were altered such as hexokinases, phosphofructokinase and pyruvate kinase. These changes are associated with alterations in the tricarboxylic acid cycle. Pathway analysis using Gene Set Enrichment Analysis suggest that metabolic pathways including, glycolysis, biosynthesis of cholesterol, glucose and pyruvate metabolism and mevalonate pathways were altered after 26 d of repeated exposure to BaP. The gene expression and pathway alterations found indicate that BaP induced disturbances in energy metabolism, which may be involved in cancer promotion.These results constitute new insights on the mechanism of action of BaP in a non-model organism (tilapia).

**MP182 Chemical fingerprinting of polycyclic aromatic compound sources in sediments using two dimensional gas chromatography mass spectrometry**

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Polycyclic aromatic compounds (PACs) are ubiquitous contaminants in the environment and many of them have been identified as known carcinogens. The associated toxicity of PACs makes monitoring and identification of sources of PACs in the environment important for assessing exposure to humans and wildlife. PACs sources in the environment include biogenic, petrogenic and pyrogenic and are generally a mixture of multiple sources mixed in environmental monitoring matrices. Most monitoring programs only analyze the PACs include the 16 EPA parent PAHs. When including all other unsubstituted and substituted PACs, there are thousands of potential congeners which could be measured to assess both toxicity and source allocation. In this study, 60 river sediment samples obtained from all across southern Alberta, Canada were analyzed by a gas chromatography-tandem mass spectrometer (GC-MS/MS) and two-dimensional high-resolution time of flight mass spectrometry (GCxGCHRToFMS) for comprehensive PAC chemical fingerprinting. Chemical fingerprinting helps distinguish between different sources of PAH in the environment. Data collected by these two techniques was statistically analyzed to determine the chemical patterns (fingerprints) of the predominant sources in the river systems. In addition to the conventional use of native PAHs for source identification, patterns of individual alkyl PACs identified using the GCxGCHRToFMS were also investigated to determine if additional source designation could be determined. Recent research has shown that these individual alkyl PACs can be more toxic than their unsubstituted analogues. Therefore, this information can aid in the evaluation of potential risk and used in risk assessment. Preliminary evaluation of data shows dominant petrogenic sources from coal type sources. In addition, the identification of high creosote like signatures in urban areas. On-going data analysis obtained from the GCxGCHRToFMS system will further suggest new patterns, PAH compound associations and source identification based on individual isomer of alkylated polycyclic aromatic compounds.

**MP183 Deuterated Phenanthrene Metabolites as Probes for the Metabolism of Carcinogenic PAHs in Humans: Phenotyping, Genotyping and Human Health Risk**

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Individual susceptibility plays an important role in cancer development in humans exposed to chemical carcinogens, thus identification of high-risk individuals is critical in cancer prevention and therapy. Benzo[*a*]pyrene (BaP), an important polycyclic aromatic hydrocarbon (PAH) in tobacco smoke and the environment, is considered carcinogenic to humans by the International Agency for Research on Cancer. BaP requires metabolic activation by the diol epoxide pathway to exert its carcinogenic effect, and this process differs greatly among individuals, who can't be identified based on exposure measurements only. In this study, we used deuterated phenanthrene ([$D_{10}$]Phe), a non-carcinogenic PAH with structural features and enzymology profile similar to that of BaP, as a probe substrate. The bioactivation of Phe bythe diol epoxide pathway yields the urinary metabolites phenanthrene *dihydrodiol*(Phe-Diol), phenanthrene tetraol(PheT) and phenanthrene quinone (PheQ), whereas phenanthrols (HOPhe) signify detoxification. A previous prospective epidemiological study has confirmed the statistically significant relationship between urinary levels of PheT and lung cancer risk. PheQ has been associated with the generation of reactive oxygen species (ROS) and oxidative damage, while Phe-Diol is the precursor of PheT and PheQ. We administered [$D_{10}$]Phe, with FDA approval, to smokers (N=101, 10 μg oral dose) and nonsmokers (N=87, 1 μg oral dose) and quantified the excretion of its metabolites mentioned above in urine.We have shown that this metabolism phenotyping approach is practical, accurate and precise. There are great variations among subjects in conversion of [D10]Phe to [D10]Phe-1,2-diol (31-fold/497-fold in 10 μg/1 μg dose group), [D10]PheT (51-fold/84-fold) and [D9] HOPhe (33-fold/84-fold). We hypothesize that the individuals who carry out higher ratio of bioactivation metabolites ([D10]Phe-Diol+[D10] PheT+[D8]PheQ) to detoxification metabolites ([D9]HOPhe) are at higher risk for lung cancer. Oral cell genotyping of GSTs were also determined to explore genetic causes of individual differences. The results of this innovative and unique study will vastly expand our understanding of metabolism of carcinogenic PAH in humans, thus providing new insights for human health risk assessment and lung cancer prevention.

**MP184 Effects of polycyclic aromatic hydrocarbon exposure on Gulf toadfish (*Opsanus beta*) and resiliency to co-stressors**

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Polycyclic aromatic hydrocarbons (PAHs) are a ubiquitous class of contaminants associated with natural geologic petroleum deposits, and pyrogenic origins, both natural and anthropogenic (forest fires, volcanic releases, contrasted with oil spills, and combustion of petroleum and coal). Because of widespread atmospheric deposition, urban runoff, and occasionally very intensive release, such as during the Deepwater Horizon (*DWH*) oil spill, PAHs are an important class of contaminants of concern. Exposure to PAHs can be a stressor to fish; however, the response at the level of the hypothalamic-pituitary-interrenal (HPI) axis is complex. If a stress response is induced by PAH exposure, which initiates a greater production of glucocorticoids, a suite of responses follows. Two such responses examined in this work are carbohydrate metabolism and immune cell mobilization. Glucocorticoids often increase gluconeogenesis. Greater glycogen breakdown in fish has been associated with poorer body condition factors. We hypothesize that stress associated with PAH exposure will favor carbohydrate metabolism, resulting in increased gluconeogenesis, increased plasma glucose, and decreased condition factors relative to controls. The initial stress response from PAH exposure may not be deleterious to the individual, however, continuous PAH-induced stress may have a supressing effect on the immune system. Furthermore, there is a growing weight of data in the literature that chronic exposure to PAHs may interfere with the vertebrate immune response. This would reduce an organism's ability to cope with parasites, wounds, and opportunistic infections, all of which may reduce survival and fitness. We hypothesize that metabolic and immune changes induced by PAH exposure will be exacerbated by additional stressors such as simulated predator pursuit. This investigation exposed Gulf toadfish (*Opsanus beta*) to relatively low concentrations of PAHs (lower ppb range) for 7 d, followed by a depuration period of equal length. *O. beta* tissues were sampled at several timepoints during exposure (0 h, 4 h, 8 h, 24 h, and 3 d, 7 d) and parallel timepoints during depuration. Metabolic change was measured as gene expression (GCG, PYGL, GLUT1), plasma glucose, body condition factor, and hepatic-somatic index. Immune response was quantified by gene expression (CD4, TNFa, Interleukin), and immune cell counts.

**MP185 Effects on Buoyancy and Energetic Demands in Mahi Embryos Co-exposed to Crude Oil, High Temperature and UV Radiation**

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The timing and location of the 2010 *Deepwater Horizon* (DWH) incident within the Gulf of Mexico resulted in crude oil exposure of many commercially and ecologically important fish species, such as mahi-mahi (*Coryphaena hippurus*), during their sensitive early life stages (ELS). ELS mahi develop rapidly and hatched larvae are presumed to float and remain in the upper layers of the water column. In a laboratory setting, mahi embryos are positively buoyant for the duration of their development, up until the period directly preceding hatch, where they become negatively buoyant. Therefore, ELS mahi may be directly exposed to the cardiotoxic tricyclic PAHs dominating the oil slicks in surface waters for a significant portion of their developmental period. Further, these embryos are transparent and are likely also exposed to simultaneous stressors occurring in surface waters such as increased temperature and ultraviolet (UV) radiation. The maintenance of buoyancy in teleost fish is critical to survival; and aids in promoting dispersal by facilitating drift through ocean currents and positioning newly hatched larvae in the upper water columns where planktonic food is plentiful. Crude oil exposure combined with environmentally relevant stressors such as UV radiation and/or increased temperature induced early onset of negative buoyancy in mahi embryos. Further, premature negative buoyancy was coupled with increased oxygen consumption rates, faster sinking rates and significant energy depletion, likely resulting in detrimental consequences for these developing fish. Recovery of positive buoyancy was observed once the UV exposure was terminated and UV-exposed embryos did not become negatively buoyant again until right before hatch and in unison with control embryos. These results point to a behavioral response, in which embryos avoid UV exposure by sinking down the water column, implying that embryos can sense UV light and dynamically control buoyancy. This research was made possible by a grant from The Gulf of Mexico Research Initiative. Grant No: SA-1520; Name: Relationship of Effects of Cardiac Outcomes in fish for Validation of Ecological Risk (RECOVER).

**MP186 Endogenous effects in embryos of Japanese medaka exposed to oxygenated polycyclic aromatic hydrocarbons**

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Oxygenated polycyclic aromatic hydrocarbons (oxyPAHs) are directly discharged into the atmosphere with exhaust gas from diesel engine automobiles and industries, and also generated through photo-oxidation and/or microbial metabolism of parent polycyclic aromatic hydrocarbons (PAHs). Because oxyPAHs have diversity of forming process, they distribute widely in environment, especially around urban area, and this is not exception in aquatic environment. Previously, fish embryos exposed to oxyPAHs were caused malformation similar with blue-sac syndrome. In this study, we examined the endogenous effects in embryos of Japanese medaka (*Oryzias latipes*) exposed to oxyPAHs such as acenaphthenquinone (ANQ), 7,12-benz(a)anthracenquinone (BAQ), 1,4-Naphthoquinone (NAQ), and 9,10-Phenanethrenequinone (PHQ). Embryos were exposed as following; 15, 150, and 1500 µg/L of ANQ; 2,20, and 200 µg/L of BAQ; 1, 10, and 100 µg/L of NAQ; and 1.5, 15, and 150 µg/L of PHQ. Solvent control group, which embryos were reared in oxyPAHs free freshwater added DMF, was also established. Then the variations with activity of ethoxyresorufin-O-deethylase (EROD), oxidative stress with generations of lipid peroxide (TBARS) and carbonyl protein, production of adenosine triphosphate (ATP), and amount of ammonia ion were individually examined in embryos with their development. EROD activity increased with increasing exposed concentration of all oxyPAHs. Additionally, oxyPAHs were actively generated ATP until 4 days post-fertilization (dpf), but significantly decreased after that. These were resulted perhaps fish embryos had continuously required the energy generation, because oxyPAHs were recognized as xenobiotics, and embryos could need the excess energy to metabolize and eliminate them. TBARS and carbonyl protein are biomarker of oxidative stress, and these were increased depending on increase of the exposure concentrations. These results suggest that oxyPAHs can cause oxidative stress in embryos. Moreover, they elevated even ammonia in embryos, embryos were expected to producing the large amount of ammonia with much ATP consumption.

**MP187 Exploring Mechanisms of Cardiotoxicity in Crude Oil Exposed Fish**

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During the spring and summer of 2010 the Deepwater Horizon (DWH) oil rig released over three million barrels of crude oil into the Gulf of Mexico. As the oil dispersed it contaminated ecosystems that support numerous Gulf species including mahi-mahi (*Coryphaena hippurus*) and red drum (*Sciaenops ocellatus*). The timing of the spill coincided with the spawning of many marine species in the region and previous studies have shown that developing fish embryos are particularly sensitive to oil exposure. Numerous abnormalities due to crude oil exposure have been documented in fish early life stages, including cardiotoxicity; however, the molecular mechanisms that cause these phenotypes are not well understood. Previously, RNA sequencing was used to evaluate the transcriptomic profiles of oil exposed red drum and mahi-mahi. Subsequent mRNA analysis not only identified cardiotoxicity, but also cholesterol biosynthesis as potential targets in both species. To determine if the observed changes in gene expression have physiological relevance, mahi-mahi and red drum embryos were exposed to slick oil high-energy water-accommodated fractions (HEWAFs) until 96 hours postfertilization (hpf) and 72hpf, respectively. Total cholesterol was quantified in the resulting larvae using enzyme linked absorbance assays and indicated a trend toward reductions in both species. Immunohistochemistry was subsequently used in the zebrafish model to visualize changes in cholesterol concentrations in larval fish and was consistent with overall reductions particularly in the heart. Understanding the mechanisms involved in producing the phenotypes associated with oil exposure will contribute to assessing the ecological risk of oil spills on fish populations. This research was made possible by a grant from The Gulf of Mexico Research Initiative. Grant No: SA-1520; Name: Relationship of Effects of Cardiac Outcomes in fish for Validation of Ecological Risk (RECOVER).

**MP188 Fish embryotoxicity changes with the weathering of a conventional medium crude oil using a wave tank approach**

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For several years now, CanmetENERGY's Devon Research Centre (Canada) has been performing experiments in a 1200-L wave tank examining the physical and chemical behaviours of various crude oil/water mixtures under varying temperature regimes with set wave mixing energy. A test initiated in November of 2017 used a sweet medium crude with air and water temperatures of 15 °C. Water samples were taken from the wave tank five times during a 28-day weathering experiment (at day 1, 6, 14, 21, and 28) and were used to perform toxicity exposures using fathead minnow embryos (*Pimephales promelas*). For each sampling time, newly fertilized embryos were exposed to a serial dilution of water accommodated fractions (WAFs), to non-contaminated river water (used to generate WAFs), and to aquatic facility water control. Embryos were raised until hatching, half were harvested for morphological assessment and biomarker analysis. While mortality was not significantly altered by the oil treatments, both malformation occurrence and severity showed strong concentration-responses with all the sweet medium crude treatments. Preliminary data suggest that days 14, 21, and 28 were the most toxic fractions to the fish embryos. Complementary chemistry analysis confirmed the changing concentrations of total polycyclic aromatic compounds (TPACs), volatile organic compounds, and C10-C50 with time. Noteworthy, there was an increase of oxidative compounds measured as oil weathered with time suggesting that these oxidative compounds could explain the additional toxicity observed at days 14, 21, and 28. Molecular biomarker analysis for *cyp1a* in whole larvae is ongoing. This study demonstrates the importance of modulating environmental factors, such as wave mixing energy, to best predict aquatic toxicity.

**MP189 Is cardiomyopathy a more sensitive mode of action than narcosis for polycyclic aromatic hydrocarbons (PAH) toxicity?**

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There has been a growing interest in the sublethal effects of petroleum hydrocarbons, and one of the best-studied groups of compounds are the tricyclic (3-ring) polycyclic aromatic hydrocarbons (Tri-PAHs) that have been observed to elicit cardiac abnormalities in exposed fish embryos. These developmental affects have been discussed in the injury assessments of biological resources following several oil spills. There are currently uncertainties in the application of the laboratory studies in literature to quantifying adverse effects (injury) and assessing damages (lost value resulting from injuries) in the Natural Resource Damage Assessment (NRDA) process. To address part of this uncertainty, in this paper we compare the effects concentrations for acute narcosis with those for developmental deformities in Medaka (*Oryzais latipes*) for selected Tri-PAHs. We also discuss how this information can be incorporated into oil spill models to calculate deformity rates in fish following exposure to petroleum hydrocarbons.

**MP190 New methods for legacy problems: Modeling approaches to predict and evaluate chemical transformations of complex environmental mixtures**

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants with many natural and anthropogenic sources. Wildfires, fossil fuel extraction and use, burning of wood-derived fuels for home heating and cooking, and even food processing release a complex mixture of PAHs into air, water and soils. Once released, these substances can undergo further biological transformations mediated by microbes, fungi and higher organisms. A number of PAHs have been identified as hazardous, including the well-known carcinogen benzo[a]pyrene. Yet much less is known about which particular PAH mixtures are likely to form given a specific set of environmental and ecological conditions, nor which PAH mixtures may contain particularly toxic compounds. Experimental analysis of PAH identity and relative concentrations in complex environmental matrices is difficult and expensive, despite recent advances in non-target analyses using mass spectrometry. Since parent compound and transformation products may exhibit different degrees of solubility in water and organic solvents, for instance, different mixture components may require LC-based or GC-based methods. Here, we propose a novel framework of complementary modeling and data science approaches to prioritize PAH compounds, degradation pathways, and their products. This framework serves two important purposes: to narrow the field of relevant chemicals to be pursued by analytical means (i.e., leading to semi-targeted analysis) and to gain insight into the environmental conditions and parent structures most likely to lead to toxic compounds.

## One Health in Action – Tangible Applications for Problem Solving and Coalition Building

**MP192 Impacts of Climate Change on the Ecotoxicology of Triclosan on the Estuarine Grass Shrimp, *Palaemonids pugio***

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Pharmaceuticals and personal care products (PPCPs) such as antimicrobial agents are one of the major contaminants of emerging concern (CECS), which are used by both humans for medical treatment and by agribusinesses to enhance the growth or health of livestock. This study evaluated the effects of increasing temperature and salinity associated with climate change conditions on the ecotoxicology of antimicrobial agent, Triclosan, (TCS)[5-chloro-2(2,4-dichlorophenoxy)-phenol], which is also known as Irgasan, which is an antibacterial agent that is widely used in soaps, toothpastes, skin creams, first-aid products, and plastics. Triclosan modes of action (resistance) or toxicity have been showed in microbes and some aquatic organisms but are not yet fully understood in terms of changing environmental conditions associated with climate change such as increasing temperature and salinity. Grass shrimp *Palaemonetes pugio*, are common inhabitants of salt marshes along the Atlantic and Gulf coasts of North America, often comprising up to 56% of pelagic macrofauna in estuarine tidal creeks and are considered a keystone species for risk assessment in coastal ecosystems. Survival of adult grass shrimp, *Palaemonetes pugio,* exposed to triclosan under standard(e.g. 20$^{o}$C, 20 Psus) and increased temperature and salinity (30$^{o}$C. 35 Psus) as a simulation of potential climate change effects, was assessed. The nominal test concentration of Triclosan ranged from 0.056-1.00 mg/L, plus a seawater control (0.1 % acetone carrier) and all tests were run in triplicate using instant ocean. Survival and behavior were the two endpoints noted in each test. Results indicated that the LC50 for Triclosan under simulated climate change conditions was more toxic, with a 96h LC50 of 0.32mg/L (CL = NC-2296.425 mg/L /L) compared to an LC50 of 0.58mg/L (CL = 0.562-0.607 mg/L) under standard conditions. These results indicate that climate change conditions must be assessed when evaluating the environmental hazards of a chemical contaminant to fully assess current and future environmental risks. As long as, consumer demand for antimicrobial products is anticipated to grow, probable prevalence of TCS increases with many concerns that the product might be harmful to human health and the environment, particularly under future climate scenarios.

**MP193 The Lancet Commission on Pollution and Health: Findings, One Health Perspectives, and SETAC Opportunities**

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Last year the Lancet Commission on Pollution and Health was released. As one of the report co-authors, here I discuss the findings of our work and also examine these through a One Health lens (e.g., parallel research on environmental quality). The Commission addressed the full human health economic costs of air, water, and soil pollution. Stark conclusions were drawn including, for example, pollution is responsible for 9 million premature deaths per year (3-times more than AIDS, malaria, and TB combined), more than 90% of these deaths are released in low- and middle-income countries, and that it may reduce the GDP of countries by up to 2%. Furthermore, these estimates (and many more), likely underestimate the true costs of pollution owing to tremendous data gaps that remain. The Commission also pointed to several success stories that highlight that solutions and strategies can have co-benefits for the environment, health, and the economy. The Commission focused strictly on human health though similar questions can be asked about environmental quality. Here, as I systematically break down the Commission's report, I will take a One Health approach and provide examples from studies that are concerned with environmental quality and animal health (e.g., how many fish and wildlife prematurely die from exposure to pollution? What are the economic costs of this?). I will conclude with some recommendations for SETAC members based on our experiences with the Lancet Commission.

**MP194 Kewaunee County, Wisconsin: Using a "One Health" Approach to Investigate Multidimensional Aspects of Antibiotic Resistance**

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Surface water ecosystems are a dynamic component of the biosphere, providing food, drinking water, and diverse animal habitats. Despite the importance of surface waters, human activities have significantly altered their health, contributing multiple classes of contaminants to these waterways. In agricultural areas, manure fertilized cropland is a primary source of contamination and is a particular problem in areas of concentrated livestock farming as these farms utilize manure fertilization for convenient and economical waste management. Research on the impact of manure runoff on surface water ecosystems has focused on nutrient loads and fecal bacteria; however, emerging biological contaminants including antibiotic resistant bacteria (ARB), antibiotic resistance genes (ARGs), and pathogens have not been well studied. Here, we address this gap by using a "One Health" approach wherein we study animal, environmental, and human facets of contaminated surface water ecosystems. Using culture-based, molecular, and sequencing approaches, we have conducted a year-long monitoring study to determine the levels of ARB, ARGs, pathogens, and chemical contaminants in Kewaunee County, Wisconsin, an area dominated by concentrated livestock farming and manure crop fertilization. Results indicate pathogens and antibiotic resistant *Escherichia coli* are widely abundant in Kewaunee County surface waters, sediments, manure, and clinical isolates, including the presence extended-spectrum beta-lactamase resistance in all sample types. Additionally, environmental and manure *E. coli* isolates display significantly higher levels of resistance to beta-lactam and cephalosporin antibiotics compared to clinical isolates. These results suggest that surface water systems may serve as environmental reservoirs for antibiotic resistance to drugs of human importance. Potential pathogens from genera including *Legionella, Pseudomonas, Flavobacterium, and Burkholderia* were identified in environmental samples throughout the year-long study. Additionally, temporal patterns of ARG contamination have been identified and are primarily associated with timing of manure waste on crop fields. Together, these data provide a glimpse into the complexities of antibiotic resistance and suggest an environmental connection between livestock antibiotic resistance and human health.

## Fate of Organic Contaminants in Aquatic Invertebrates and Their Environments – Measurement, Modeling and Risk Management

**MP195 Do Bioaccumulation Metrics Depend on Exposure Concentration?**

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Bioconcentration factor (BCF), biota-soil/sediment accumulation factor (BSAF), and bioaccumulation factor (BAF) are used to quantify the potential of organic compounds to accumulate in various organisms under different laboratory or field exposure scenarios. These factors or concentration ratios are currently the standard metrics for bioaccumulation and have been widely adopted in scientific, risk assessment, and regulatory contexts. Since these metrics are essentially the ratio of concentration in the biota over that in the exposure medium, a critical underlying assumption of these standard metrics is that they do not vary with exposure concentration or chemical activity. However, standard bioaccumulation measurements are often conducted at concentrations higher than at field conditions. This study attempts to examine this assumption by reviewing bioaccumulation data available in literature. With a main focus on worm related bioaccumulation data, experimental bioaccumulation factors reported across different exposure media including soil, sediment, and aqueous solution are collected and reviewed. More than 100 chemical-medium combinations were found and analyzed for dependence on exposure concentration. Results suggest that the standard bioaccumulation factors tend to decrease with increasing exposure concentration across a wide variety of chemicals. This suggests that existing bioaccumulation metrics may be inadequate, and that standard laboratory-based bioaccumulation measurements may understate the bioaccumulation risk at the field. Various causes and reasons for this dependence are examined.

**MP196 Polychlorinated Biphenyl Contamination on the Island of Unalaska**

*E. Adams, Northern Arizona University*

Polychlorinated biphenyls (PCBs) are a group of man-made, hydrophobic organochlorines that persist at highly toxic levels in the environment and biomagnify within food webs. Although banned, their continued release from pre-banned products and persistence in the environment impact human and wildlife health. PCBs are transported to the Arctic via global distillation and biomagnify to high levels in the lipid- rich food web. Thus the long-range transportation capacity of PCBs can affect food webs far from the area of release. In addition, the Arctic contains thousands of Cold War military installations, many of which are also a local source of PCB contamination. Few studies have investigated local source PCB contamination from formerly used defense (FUD) sites in the Aleutian/Bering Sea region of Alaska. PCBs have the ability to modify or suppress thyroid, reproductive and immune function. Exposure can reduce cognitive function and increase the risk of developing cancer, hypothyroidism and a host of other negative health effects. Human and animal exposure occurs via ingestion of contaminated food. PCBs were extracted from aquatic organims, focusing on important subsistence foods (i.e. salmonids, blue mussels) for the Qawalangin Tribe of Unalaska. Samples were analyzed using a modified QuEChERS method and congener-specific PCB profiles were used in target species to distinguish between point source contamination and global distillation of PCBs. Elevated levels of PCBs were found in blue mussels of two FUD sites. Levels at these two sites exceed the safe consumption limits set forth by the EPA giving supporting evidence toward the prioritization of FUD site remediation in these areas. PCB level data provided also allows the indigenous people of the area to make informed decisions about what subsistence foods are safe for consumption.

**MP197 Kinetic Sorption and Bioaccumulation of Polycyclic Aromatic Hydrocarbons in Marine Plankton Food Chain**

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Bioaccumulation and distribution of polycyclic aromatic hydrocarbons (PAHs) over different amounts of exposure time were investigated in the plankton food chain including phytoplankton and zooplankton. The simulated plankton food chain was using phytoplankton (*Tetraselmis chuii*), rotifers (*Brachionus* sp.), and copepods (*Apocyclops* sp.) cultured in a gas purging system with a steady supply of PAHs for 7 days in this study. The results show that PAH accumulation in plankton can be roughly divided into three sections: 0.2-1 hours, 1-24 hours, and 24-168 hours. The PAH concentrations in plankton varied greatly over the 0.2-1 and 1-24 hour time intervals, then approached study-state at 24-168 hours exposure. The low molecular weight PAHs (ACN, AC) were found at significantly higher levels in copepods than in rotifer and phytoplankton, but the high molecular weight PAHs (FA and PY) were found at significantly higher levels in phytoplankton, indicating that plankton might have selectivity towards PAHs. In principal component analysis (PCA), the plankton could be separated significantly into phytoplankton and zooplankton. Parts of the PAH accumulation found in rotifers and copepods were similar, demonstrating that PAH composition in plankton might be affected by trophic levels. All PAHs demonstrated significantly linear relationships between bioconcentration factor (BCF) and PAH hydrophobicity ($K_{ow}$) in plankton, however the different linear regression slopes of log BCF and log $K_{ow}$ between phytoplankton, rotifer and copepod, suggested that the plankton have different pathways of PAH accumulation.

**MP198 In Vivo and in Vitro Biotransformation of Chlorinated Polyfluoroalkyl Ether Sulfonate in Rainbow Trout**

*S. Yi, Univerisity of Toronto / Department of Chemistry*

Increasingly raised concerns on perfluorooctane sulfonate (PFOS) lead to its global voluntary phase-out initiatives by producers in consideration of its biological toxicities and persistence. In the meantime, 6:2 chlorinated polyfluoroalkyl ether sulfonate (6:2 Cl-PFESA, trade name F-53B) has been applied as mist suppressant to replace PFOS in metal plating industry for three decades. Recently, extensive concern has been specially focused on this class of chemical due to its widespread presence in riverine water, sewage sludge, freshwater and marine organisms, atmospheric particulate matters and human serum, with comparable or higher levels to/than PFOS. The structural similarities between 6:2 Cl-PFESA and PFOS make it reasonable to assume that 6:2 Cl-PFESA may display similar bioaccumulative potential and persistence as its predecessor. Nevertheless, the inclusion of a chlorine substitution and ether linkage in 6:2 Cl-PFESA may result in more susceptibility to biotransformation, yet no reported data has focused on their potential metabolites in biota. In this study, degradation potency of 6:2 Cl-PFESA was concomitantly investigated via in vivo and in vitro tests using juvenile rainbow trout and rat S9 as the animal model, respectively. In the in vivo tests, we examined the existence of 6:2 hydrogen-substituted polyfluoroalkyl ether sulfonate (6:2 H-PFESA) in fish blood samples as a metabolite after exposure to 6:2 Cl-PFESA in the diet for 30 days, followed by 40 days of depuration. In vitro tests further confirmed the biotransformation occurrence through detection of the same metabolite within 4 hours. This study will provide an insight on 6:2 Cl-PFESA degradation mechanisms in biota, and specify the structure-dependent manner in determining biotransformation potentials.

## Addressing Beneficial Use Impairments at Great Lakes Areas of Concern: Scientific Approaches that Lead to Restoration

**MP199 A Toolbox for Assessing Beneficial Use Impairments in Fish-Eating Birds at Areas of Concern Under the Great Lakes Restoration Initiative**

*K. Grasman, Calvin College / Biology; L.L. Williams, M. Annis, US Fish and Wildlife Service; J. Moore, US Fish and Wildlife Service / Interior*

Significant concentrations of persistent organic pollutants (POPs) including polychlorinated biphenyls (PCBs), chlorinated dioxins and furans, and organochlorine pesticides have contaminated the Great Lakes since their introduction during the 1940s-60s. Studies from the late 1960s onward have shown that fish-eating birds of the Great Lakes are excellent sentinel species for assessing and monitoring effects of contaminants including reproductive problems, deformities, and immune suppression. Two wildlife-related Beneficial Use Impairments (BUIs) at Areas of Concern (AOCs) are recognized by the Great Lakes Water Quality Agreement: 1) bird or animal deformities or reproductive problems and 2) degraded fish and wildlife populations. A current question under the Great Lakes Restoration Initiative (GLRI) and other federal and state AOC programs is whether these impairments continue. Furthermore, more recently bioaccumulative contaminants of emerging concern (CECs) have been found in Great Lakes wildlife, raising the question of whether these CECs are associated with reproductive, health and (or) population-level effects. This talk will present the tools and key results of a monitoring program studying fish-eating birds to reassess these BUIs and potential associations with legacy POPs and CECs in the Saginaw Bay and River Raisin AOCs during 2010-18 under the GLRI and AOC programs of the US Fish and Wildlife Service and US Environmental Protection Agency. Embryonic nonviability through late incubation in herring gulls in the Saginaw Bay (6.8%) and River Raisin (8.6%) AOCs was significantly higher than at the reference site (3.2%). Infertility was the primary cause of nonviability at the reference site. Elevated infertility and mortality contributed to nonviability in contaminated sites. Deformities associated with PCBs and PCDDs were found in several embryos and chicks only at AOCs. Chick productivity in Caspian terns in Saginaw Bay was significantly below that of reference sites. In the River Raisin AOC, productivity of gull chicks was very poor in 4/8 years, with complete reproductive failure during 2010. The mean phytohemagglutinin (PHA) skin response for T-cell mediated immunity was suppressed 52-55% in gull chicks at both AOCs and 46% in terns and 39% in herons in Saginaw Bay. This monitoring program has demonstrated continuing wildlife BUIs at two AOCs and provides a model for assessments that could be applied at other AOCs to support management decisions.

**MP200 Assessing the suitability of the Bad River as a reference for the St. Louis River AOC using geochemistry, biotic condition, bioaccumulation of mercury**

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The St. Louis River Area of Concern (AOC) was designated in 1987 due to Beneficial Use Impairments (BUIs), including “Restrictions on Fish and Wildlife Consumption” (BUI 1) from historical pollutants. Minnesota and Wisconsin posted fish consumption advisories for the St. Louis River, in part, because fish have elevated mercury concentrations. Mercury bioaccumulation in the food web can be from legacy sources in sediment, as well as present-day ambient sources (from both local and regional sources). In 2017 a study was conducted to achieve four goals to support the understanding of Hg sources in the AOC : 1) Compare mercury methylation and bioaccumulation by characterizing the macroinvertebrate assemblages, geochemistry, ecosystem type, and food web structure between the AOC and the reference location (Bad River); 2) Compare age- and size-specific mercury residues in select fish species between the AOC and Lower Bad River; 3) Trace food web pathways and identify mercury sources in select fish species using stable isotopes of carbon and nitrogen, and of mercury, respectively; 4) Develop a mercury-specific BUI restoration target for the AOC based on the long-term, post-remediation projected change in fish mercury residues based on the inferred differences in mercury-source bioaccumulation between the AOC and Lower Bad River. For this presentation, we will focus on Goals 1 and 3. For each location, up to five samples each were collected for mayfly (Hexagenia) larvae, odonate larvae, riparian spiders, and a composite benthic invertebrate assemblage samples using multi-plate artificial substrate samplers. For Biotic condition and mercury tissue concentrations in macroinvertebrates, a new multi-plate artificial substrate device was employed. Thirty-nine total sites were sampled with 25 sites in the AOC and 14 sites on the Lower Bad River. Sixteen samplers were deployed at each site and left to colonize for 4 weeks. When the samplers were retrieved, 12 of 14 were dissembled, scraped, sieved then picked for biomass. Two samplers were set aside for identification and metabarcoding to assess similarity in benthic communities. Macroinvertebrate assemblage, water, sediment and biota mercury, plus other water quality analyses will be presented and discussed.

**MP201 Polychlorinated Biphenyl (PCB) Inventory and Assessment in the Great Lakes Region**

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As the consummate legacy persistent organic pollutant (POP), polychlorinated biphenyls (PCBs) have long been a chemical of concern for their persistence, toxic and bioaccumulative effects. They were primarily used as dielectric fluids in electrical transformers, capacitors and other equipment. These primary sources of PCBs are on-going sources of accidental releases into the environment, which is of particular concern near bodies of water. Canada has committed to phasing out PCBs by 2025 as a signatory of the Stockholm Convention. The US does not have regulations on phasing out in-use PCBs, although national regulations stopped new production and new uses in 1974. We investigated the extent of regulatory compliance by compiling an inventory of PCBs in Ontario and US states bordering the Great Lakes. Mass of pure PCBs was estimated using the data from the Canadian Federal and Provincial (Ontario) PCB databases, and the USEPA databases. We also compared the PCB inventory for Toronto between 2006 and 2016 to assess the level of compliance with the Federal regulations. We found that the in-use and stored mass of pure PCBs in Toronto has decreased within 10 years from 420 tonnes to 0.18 tonnes. This demonstrates effective progress on regulation compliance, especially in the downtown core where PCBs were used extensively in transformers of large skyscrapers built in the 1960s and early 1970s, and in electrical infrastructure such as the public transportation system. In Ontario, 2.7 tonnes of PCBs in-use and in storage remain: they are widely distributed across the heavily populated shores of Lakes Ontario and Erie. Ontario also holds 88 tonnes of PCB waste, mostly consisting of contaminated soil/gravel. We estimated that the eight states bordering the Great Lakes (Illinois, Indiana, Michigan, Minnesota, Ohio, New York, Pennsylvania, and Wisconsin) have ca. 1900 tonnes of pure PCBs reported in-use or in storage from 1998-2011, which could be underestimated as the available registry is incomplete and out-of-date. The assessment of PCB stocks in the Great Lakes region suggests that passing binding national regulations to track and remove in-use and in storage stocks has been successful. In terms of Stockholm POPs, unfortunately this may be an exceptional case as PCBs are unique in mostly being used in contained infrastructure located in facilities that can be monitored, in contrast to legacy pesticides and flame retardants that have diffuse sources.

**MP202 A Contaminated Legacy: PCBs in Michigan’s Areas of Concern**

*E. Shaw, Michigan Technological University; N.R. Urban, Michigan Technological University / Civil and Environmental Engineering; M. Priyadarshini, Michigan Technological University*

Polychlorinated biphenyls (PCBs) are ubiquitous contaminants worldwide and are the most frequent contaminant at US and bi-national areas of concern (AOC). To address the hazardous legacy throughout the Great Lakes basin, many sites are designated as Areas of Concern (AOC) based on their impaired use (i.e. fish consumption advisories). Statewide fish consumption advisories are indicative of the breadth of the contamination problem, which is exacerbated by above average fish consumption for people living within the Great Lakes basin. Using Michigan’s fish contaminant monitoring data, this work evaluated existing evidence to answer the questions: *do existing practices enable accurate and reliable evaluation of PCB contamination at AOC sites,* and *have remediation efforts been effective?* Paired t-tests indicate significant differences in PCB concentrations between AOC and non-AOC sites for carp, largemouth bass, and walleye but not for smallmouth bass. Among the four fish species, only in carp are PCB concentrations significantly different from the other species. In some cases, toxicity of fish at both AOC and non-AOC sites exceeded the EPA’s dioxin-like compound reference dose in spite of all dioxin-like and most mono-ortho substituted PCB congeners being in < 50% of samples. The nuanced differences between AOC and non-AOC sites support recommendations for altering the current monitoring strategies to evaluate PCB contamination and efforts to remediate such sites. Time trends based on congener data were difficult to establish due to many sites having less than three years of data. Combined totals (Aroclor totals and congener totals) are used to calculate longer-term time trends but this approach ignores the discrepancies between the two methods. Furthermore, these results suggest that monitoring strategies may be inappropriate for evaluating the effects of remediation. Thus, the assumption that AOCs are significantly more contaminated than non-AOC sites may be flawed. Restructuring sampling and risk assessment methods can help us to address environmental justice issues surrounding contaminant exposure through fish consumption.

## Per- and Polyfluoroalkyl Substances: Recent Advances and Future Directions

**MP203 Short-Chain Fluorotelomer-based PFAS: Recent Advances in Toxicology, Biodegradation, Water Remediation, Assessment of Alternatives and Value-in-Use**

*S. Korzeniowski, FluoroCouncil / BeachEdge Consulting; J. Bowman, FluoroCouncil*

Per- and polyfluoralkyl substances (PFAS) is a term that describes a wide and diverse array of chemistry containing fluorine and carbon. It is noteworthy that the chemistry and properties are vastly different across the various PFAS categories and classes. The primary focus of this presentation will be on the fluorotelomer-based products with six or less fluorinated carbons (“short chain”) including fluorosurfactants, side-chain fluoropolymers and potential degradation products such as perfluorohexanoic acid (PFHxA). Fluorotelomer-based products can be in either the polymeric or non-polymeric PFAS categories. Within the non-polymeric PFAS category, fluorotelomer-based surface active agents (e.g. “fluorosurfactants”) are used in complex multi-component formulations such as cleaning products, paints, coatings and Aqueous Film Forming Foams (AFFF). The non-polymeric fluorotelomer-based products provide superior surface wetting and leveling properties and unmatched AFFF fire-fighting performance on high-hazard Class B (i.e., hydrocarbon & polar solvent liquids) fires. Within the polymeric PFAS category are the side-chain fluorotelomer-based repellent products that are used in carpets, paper products and textiles providing essential and critical properties on high-end performance and medical garments, work wear, first responder gear and in military uses. During the past two years there have been significant advances in short-chain toxicology studies, soil bio-degradation assessments, progress in the removal of short-chain acids from ground and drinking water, and various exposure assessments. This presentation will summarize a number of these critical and significant advances and present the results in the context of the overall risk in using the short-chain fluorotelomer-based products. In addition, we will discuss a decision wheel to help in the assessment of alternatives (risk vs. precaution), as well as challenges and success in the development of short-chain fluorotelomer-based products and an overview of their value-in-use including some critical end uses. Industry best practices or guidelines published by the FluoroCouncil and Fire Fighting Foam Coalition for use of these products will be highlighted during the course of this presentation.

**MP205 PFAS in NIST Standard Reference Materials**

*J. Reiner, NIST / Chemical Sciences Division*

Standard Reference Materials (SRMs) are homogeneous, well-characterized materials that are used to validate measurements and improve the quality of analytical data. The National Institute of Standards and Technology (NIST) has a wide range of SRMs that have values assigned for legacy organic pollutants. These SRMs can serve as target materials for method development and measurement for contaminants of emerging concern. Since inter-laboratory comparison studies have shown considerable disagreements when measuring per- and polyfluoroalkyl substances (PFAS), future analytical measurements will benefit from the characterization of PFAS in SRMs. Currently NIST has added measurements of over 15 PFAS to ten SRMs available for purchase. These natural matrix SRMs include human serum and plasma, fish tissue, soil, house dust, and domestic sludge. These SRMs are useful to the global analytical community and can serve as materials for quality assurance measurements and method development. This presentation will highlight the ten SRMs available and discuss how environmental and exposure science can benefit from their use.

**MP206 A rapid method for the analysis of perfluorinated alkyl substances in serum by hybrid solid-phase extraction**

*M. Honda, Wadsworth Center / New York State Department of Health; M. Robinson, K. Kannan, Wadsworth Center / New York State Department of Health / Division of Environmental Health Sciences*

A method for the analysis of thirteen perfluorinated alkyl substances (PFASs) in human serum was developed based on hybrid solid-phase extraction (hybrid-SPE) and ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Serum PFASs were extracted using hybrid-SPE-phospholipid cartridge after precipitating proteins and other endogenous biological interferences with 1% ammonium formate in methanol. The average intra-day accuracy (measured as percent recoveries from fortified samples) and precision of the method (measured as relative standard deviation [RSD, %] between analyses) were 88.7–117% and 1.0–13.4 %, respectively. The average inter-day precision was 2.8–6.9 %. The method was sensitive, with limits of quantification (LOQs) in the range of 0.05 to 0.09 ng $mL^{-1}$ for all 13 PFASs. The applicability of this method was tested by analyzing serum-certified standard reference material and proficiency test samples. In an hour, 100 samples can be processed by hybrid-SPE, and the instrumental run time is 5 min per sample. The developed method is rapid, inexpensive, accurate, precise, and extremely sensitive for the analysis of PFASs in human serum.

**MP207 Effect of Head Space and Reaction Time on the Quantification of Total Perfluoroalkyl Acid Precursors by Potassium Persulfate – Fluorotelomer Alcohol**

*S. Masunaga, Yokohama National University / Faculty of Environ. & Information Sci.; S. Kitaguchi, Yokohama National University / Graduate School of Environment and Information Sciences*

Per- and poly-fluoroalkyl substances (PFASs) have been receiving attention because of their persistence in the environment and global distribution. Among them, production and use of perfluorooctanesulfonic acid (PFOS) and its salts perfluorooctane sulfonyl fluoride have come to be regulated under the Stockholm Convention on POPs. The production of perfluorooctane carboxylic acid (PFOA), longer-chain homologues and their related compounds is phasing out due to the abandonment of their production by their major traditional manufacturers. However, many other perfluoroalkyl acid (PFAA) derivatives are still being produced. As they may transform into PFAAs in the environment, they are called PFAA precursors. As the precursors are numerous and diverse in their structures, it is difficult to monitor them individually. Houtz and Sedlak (ES&T, Vol. 46, p. 9342-9349, 2012) proposed a method that can determine total concentration of PFAA precursors by their oxidative conversion into perfluoroalkyl carboxylic acids (PFCAs). In this study, we evaluated this method using a volatile PFAA precursor, fluorotelomer alcohol. We selected 1H,1H,2H,2H-perfluoro-1-decanol or 8:2 fluorotelomer alcohol (8:2 FTOH) as an example of volatile PFAA precursor. This compound has 8 fluorinated carbons and a 2-carbon ethyl alcohol group. Our results indicated that head space in the reaction vessel inhibited the conversion of 8:2 FTOH to PFCAs. Complete primary degradation of 8:2 FTOH could not be accomplished even after 12-hour oxidation at 85 °C. Meanwhile, carbon chain length of produced PFCAs became shorter, namely from $C_7$

to $C_4$ through $C_6$ and small amount of $C_5$. Therefore, this method can tell only how much molarity of precursors was present in the sample and it was difficult predict the exact perfluorocarbon chain length of precursors.

**MP208 Toxicological Evaluation of 4,4,5,5,6,6,7,7,8,8,8-Undecafluoro octanoic acid (5:3 Acid)**

*R.C. Buck, S. Gannon, The Chemours Company / Fluoroproducts*

Studies were conducted to evaluate mammalian and aquatic toxicology of 4,4,5,5,6,6,7,7,8,8,8-undecafluoro octanoic acid (5:3 acid) (CAS# 914637-49-3). 5:3 acid is a metabolite of 6:2 fluorotelomer alcohol (6:2 FTOH). 6:2 FTOH is a raw material for fluorotelomer-based products. Rat oral and dermal $LD_{50}$ values were 2950 mg/kg and >5000 mg/kg, respectively. 5:3 acid produced no dermal irritation but irreversible eye irritation in rabbits, while a weak dermal sensitization response was observed in mice (LLNA), with an EC3 of 23%. A bacterial reverse mutagenicity assay and an in vivo rat micronucleus assay were negative. A 2-week rat gavage study identified red blood cells (anemia), liver (hypertrophy, focal necrosis, and increased beta-oxidation), kidney (tubular vacuolation), stomach (ulceration), and thyroid (follicular hyperplasia; of questionable relevance to humans) as target organs. Rats dosed at 600/900 mg/kg/day were euthanized *in extremis*. The NOAEL was < 30 and 30 mg/kg bw/day in males and females, respectively. In vitro metabolism was evaluated in male and female rat hepatocytes and predicted half-life in males and females of 430 and 2573 min., respectively, and identified several metabolites. Preliminary evaluations (single dose and repeat dose exposures) demonstrated that 5:3 acid exhibits biphasic pharmacokinetics. Studies demonstrated low to moderate toxicity in aquatic organisms with the following results: 72-hour EbC50 in *Pseudokirchneriella subcapitata* was 22.5 mg/ml; 90-day NOEC in rainbow trout was 9.14 mg/L; 48-hour EC50 in *Daphnia magna* was >103 mg/L; 21-day NOEC in *Daphnia magna* was 1.25 mg/L. The 90-day bioconcentration factor (BCF) measured in rainbow trout was 2.25-5.85, which supports a lack of predicted bioaccumulation in fish. 5:3 Acid, a metabolite of 6:2 fluorotelomer alcohol, is not expected to be harmful to human health or the environment at relevant concentrations.

**MP209 Binary mixtures of perfluoroalkyl acids show additive toxicity in an amphibian fibroblast cell line**

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Perfluoroalkyl substances (PFASs) are a group of persistent organic pollutants with widespread environmental distribution in soils and surface waters. Recent research shows a potential for both bioaccumulation and toxicity of these compounds in wildlife. Due to their unique physiology and life history, amphibians are at high risk for both accumulation and toxicity following environmental exposure to these chemicals. However, understanding of the adverse effects of exposure on one of the most imperiled taxonomic groups is only beginning. Here, we examine the cytotoxic effects of these chemicals, singly and in binary mixtures, on an amphibian fibroblast cell line. We tested four common PFASs: perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), perfluorohexane sulfonate (PFHxS), and perfluorohexanoic acid (PFHxA). Of these, PFOS induced cytotoxicity at the lowest concentrations, while PFHxA was least cytotoxic. Binary mixtures allowed for the construction of isobolograms to test for additivity, super-additivity (synergism), or sub-additivity (inhibition). In all cases, regardless of functional group or carbon chain length used in the experiment, mixtures were found to be approximately additive. This data suggests that considering the sum of all PFAS present may be useful when considering effects of multi-chemical exposure.

**MP210 Toxicological Response of *Chironomus dilutus* to Six Perfluoroalkyl Compounds**

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A multi-facetted bioassay study was conducted on six different perfluoroalkyl substances (PFAS) with the intent of obtaining data to evaluate relative or proportional toxicity between the substances. The six substances were selected from the EPA's third Unregulated Contaminant Monitoring Rule (UCMR3) list. Two of the substances, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), are well studied. The other four substances (Perfluorononanoic Acid [PFNA], Perfluorobutanesulfonic Acid [PFBS], Perfluorohexanesulfonic Acid [PFHxS], and Perfluoroheptanoic Acid [PFHpA]) are commonly detected in groundwater around the US, but far less is known about their toxicological potential. 10-day acute range finding tests and 20-day chronic definitive renewal bioassays were run on all six compounds using the freshwater midge, *Chironomus dilutus*. Exposure doses were established above and below environmentally relevant concentrations. Measurement endpoints included: percent larval survival; larval growth; and number of larvae, pupae, and emergent adults at test termination. Results from the 10-day acute survival tests were used to inform dosing for the 20-day chronic growth tests. Dose response curve fitting was performed for each compound. Additional statistical analysis was performed to assess the relationship between the more toxic PFOS and the other compounds. A second round of chronic testing was performed to investigate potential additive and synergistic toxicity. Organisms were exposed to mixtures containing differing proportions of the 6 compounds, based upon ratios of detected concentrations in field collected data. Results are in concert with previous work showing PFOS to be the most toxic PFAS and additive toxicity with PFHxS. Additive toxicity with other PFAS appears less clear and needs further investigation. Results of this work will be considered in conjunction with other ongoing work testing the same compounds and mixtures to look for patterns of similarity among differing classes of organisms.

**MP212 Fluoropolymers Are Unique, Low Hazard PFAS Needing Different Analytical and Regulatory Approaches**

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Fluoropolymers, such as polytetrafluoroethylene (PTFE), differ from other monomeric and polymeric per- or polyfluoroalkyl substance (PFAS) classes, such as perfluoroalkyl acids, or polymeric precursors that degrade to them. Fluoropolymers do not demonstrate the same toxicity or physical/chemical/thermal properties as other PFAS. Fluoropolymers, such as PTFE do not meet the criteria of PBT (Persistent/ Bioaccumulative/ Toxic) or vPvB (very Persistent/ very Bioaccumulative) chemical substances, nor do they meet the Persistent, Mobile and Toxic (PM or PMT) substances criteria proposed by the German Environmental Agency, Umwelt Bundesamt (UBA, 2017). As high molecular weight fluoropolymers (e.g., PTFE) are benignly persistent (i.e., not mobile, bioaccumulative or toxic), all "highly fluorinated" substances do not pose equivalent health or environmental hazards and thus should not be regulated as a single class of chemicals. Chemical analytical techniques useful for differentiating one fluorinated substance from another are readily available, reliable, and reproducible and should be employed to identify and quantify those highly hazardous monomeric per- and poly-fluoroalkyl substances (PFAS) individually, rather than techniques aggregating all fluorine containing substances into one group (e.g, total organic halogen, and total organic fluorine). Therefore, high molecular weight fluoropolymers, as a uniquely benign class of polymeric PFAS, require analytical and regulatory approaches differentiating them from fluorine-containing substances that present health and environmental hazards.

## Advances in Passive Sampling Methods: Research to Application

### MP213 In situ calibration of passive sampling methods combined with multiresidue GC and LC target screen systems

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Sampling rates (Rs) for passive samplers calculated in situ should provide the best possible approximation of time weighted average water concentrations because they account for differences in water matrix and flow conditions. In this study, Chemcatchers and POCIS passive samplers were chosen to investigate trace organic chemical residues originating from either wet weather sewage overflows or urban stormwater runoff as a part of Sydney Water's "Wet Weather Overflow Monitoring" project. First phase of this project was to conduct in situ calibration of those passive samplers – Chemcatcher and POCIS combined with multi-residue GC/MS and LC/QTOF-MS database analysis methods that together can investigate about 1,300 organic chemicals including pharmaceuticals and personal care products (PPCPs), pesticides, PCBs, PAHs, flame retardants, antioxidants, sterols such as coprostanol (faecal indicator). A set of six Chemcatchers fitted with SDB-XC/SDB-RPS disks and covered with Omnipore diffusion limiting membrane, along with a set of six POCIS containing Oasis HLB sorbent were deployed at three sites in Sydney. Every week for six weeks composite water samples from autosamplers and one set of Chemcatchers/POCIS were retrieved. Each composite water sample was composed of 100mL of water collected by the autosampler every three hours for five days of each week. Duplicates, field blanks, and laboratory blanks were added for QA/QC purpose. Water samples were extracted through SDB-XC disks or Oasis HLB, and with the passive sampler disks/sorbents were eluted with dichloromethane and acetone for GC analysis, methanol for LC analysis and analysed by AIQS GC/MS or LC/QTOF-MS database methods after addition of corresponding internal standards for each GC or LC analysis. Detected chemical concentrations in water and the mass on the passive samplers were analysed to determine whether they were appropriate for calibration. Data for the first four weeks were the most ideal to obtain sampling rates (as indicated by 42 chemicals). The cost effectiveness of passive sampling for the 1300 chemicals covered by the multi-residue methods compared to autosampling is assessed.

### MP214 Passive Sampling of Surface Waters in Northern California using Chemcatcher® for Agricultural and Urban Use Pesticides

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Passive samplers can be deployed in the field for extended periods of time, which provides data that better represents the long-term environmental conditions of the site. This study compared pesticide detections in two different passive sampler receiving phases, Atlantic HLB (Hydrophilic-lipophilic balance) and Empore SDB-RPS (Styrene divinylbenzene reversed phase sulfonate). These were used with Chemcatcher® passive sampling devices at three locations within the Sierra Nevada and Sacramento/San Joaquin Delta. The Sierra Nevada does not have much agriculture, but has its own unique pesticide applications. Unregulated or illegal Cannabis cultivators apply unreported types and quantities, including substances banned in the United States. Pesticides are also legally used in Sierran commercial timber production and for maintenance of rights-of-way. These chemicals may be entering streams at concentrations harmful to aquatic animals and plants. A pilot study utilized the Chemcatcher® without diffusion limiting membranes (DLM) to sample surface waters downstream from Cannabis and timber production sites during the fall storm season of 2016, and with DLMs in the spring of 2017. Two different DLMs were utilized: low-density polyethylene (LDPE) was used with the Atlantic HLB, and polyethersulfone (PES) with the Empore SDB-RPS. A total of eight compounds (three herbicides, four fungicides, and an insecticide degradate) were detected. To gain insight into the differences between receiving phases as well as deploying with and without diffusion limiting membranes, additional samplers were deployed for two weeks in fall of 2017 in an area with known pesticide detections (Ulatis Creek, within the Delta). A total of 27 pesticides were detected from Ulatis Creek, 6 of which were detected in the HLB, and 24 in the SDB-RPS. The membranes from the passive samplers deployed at Ulatis Creek were also analyzed, and a total of 20 pesticides were detected, 4 of which were detected in the LDPE and 20 in the PES.

### MP215 Evaluation of Equilibrium Passive Sampling Polymers for Monitoring Munition Constituents in Aquatic Systems

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A wide array of military munition constituents are present in water bodies surrounding historically deposited unexploded ordnance.A common approach for monitoring explosive constituents involves the collection of large quantities of discrete water samples combined with solid phase extraction to insure sufficient sensitivity and detection. More recently, polar organic chemical integrative samplers (POCIS) have been tested for in situ assessment of munition compounds at underwater sites. However, POCIS requires extensive calibration prior to exposure in the field (i.e., determination of sampling rates). Simple and sensitive monitoring efforts are necessary to better understand the bioavailability and potential long-term bioaccumulation and toxicity of military munition constituents in different aquatic environments. In this study, the nonpolar polymers polydimethylsiloxane (PDMS), low density polyethylene (LDPE) and polyoxymethylene (POM), typically used to measure freely dissolved ($C_{\text{free}}$) concentrations of legacy contaminants in surface water and sediment porewaters, are evaluated as equilibrium passive samplers for munition constituents. The use of POCIS sorbents including Oasis HLB, Ambersorb and Isolute Env$^{+}$ in an equilibrium sampling approach is also being investigated and compared to the performance of nonpolar samplers. All polymer materials were successfully loaded in methanol followed by solvent evaporation with the munition constituents nitroguanidine, hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), 2-amino-4,6-dinitrotoluene and tetryl serving as performance reference compounds (PRCs). Loaded polymers were exposed in batch water systems spiked with the munition constituents 4-amino-2,6-dinitrotoluene, pentaerythritol tetranitrate (PETN) and 1,3,5,7-tetranitro-1,3,5,7-tetraazacyclooctane (HMX). The release and uptake of explosive compounds was monitored in polymer and water phases over time. Preliminary results suggest that the stronger affinity of PETN to PDMS may be explained by its higher octanol-water partition coefficient (log $K_{OW}$= 3.7) as compared to other munition constituents in the water phase, and thus favored hydrophobic interactions with the polymer. The presentation provides information both on the uptake and release of explosive compounds to a variety of polymeric materials and discusses their potential for application in equilibrium passive sampling.

### MP216 Demonstration of Passive Sampling Devices for Monitoring of Munitions Constituents in Underwater Environments

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As a result of military activities, unexploded ordnance (UXO) and discarded military munitions (DMM) are present in underwater environments, which has resulted in the release of munitions constituents (MC) including the high explosives, 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), along with their primary degradation products, to the water column and adjacent sediments. This research focused on the optimization and field validation of a commercially available in situ passive sampling device, the Polar Organic Chemical Integrative Sampler (POCIS), for detection and quantification of MC in underwater environments. POCIS provide integrative, continuous sampling in situ, and ultra-low detection limits and potentially more meaningful time-weighted averaged (TWA) water concentrations, compared with more traditional sampling (e.g. grab) methods. Sampling rates for multiple MC were determined, and accounted for parameters (e.g. flow velocity, biofouling) that are known to influence POCIS uptake rate. These laboratory based efforts were followed by two unique field demonstration efforts. The first involved an uncontaminated estuarine site where a known quantity of the explosive fill material Composition B (39.5% TNT, 49.5% RDX, 1% wax) was placed. TWA concentrations for TNT and RDX ranged from 9 to 103 ng/L, with the highest concentrations nearest the Composition B source, but all samplers positioned greater than 2 m from the source resulted in non-detects. The controlled study was followed by placement of POCIS within the Live Impact Area at the former Vieques Naval Training Range at Bahia Salina del Sur (Vieques, Puerto Rico), a bay with documented high incidence of munitions items including UXO. The Vieques study design involved POCIS placement within ~6" of 15 potentially leaking UXO, and an unbiased (i.e. a grid design over a ~200 acre area) placement, also 15 locations, in the bay. When detected, TWA concentrations for TNT and RDX were observed at ultra-trace concentrations (range = 4 to 13 ng/L), except 6" from an apparent leaking munition where the TNT TWA concentration was 5.3 µg/L. All detects were at least two orders of magnitude lower than ecological risk thresholds derived from recently updated species sensitivity distributions for these constituents.

**MP217 Monitoring Neonicotinoid Insecticides with Pocis Passive Samplers in Watersheds of the Great Lakes Basin in Ontario, Canada**

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We evaluated the distribution and concentrations of a range of neonicotinoid (NNI) and structurally related insecticides in watersheds that drain into the lower Great Lakes in Ontario, Canada. Polar Organic Chemical Integrative Samplers (POCIS) were deployed in 18 watersheds during late May to late June of 2016. Grab samples were also collected in 7 of these watersheds. Sampling rates determine in the laboratory (i.e. $R_{s\text{-}cal}$) were adjusted according to the loss of Performance Reference Compounds in POCIS deployed in the field (i.e. $R_{s\text{-}field}$) to compensate for the effects of environmental conditions on sampling rates. There was generally good agreement between the time-weighted average concentrations of NNIs estimated from the POCIS and the concentrations detected in grab samples. The monitoring data indicated that the NNIs, thiamethoxam, clothianidin and imidacloprid were present in several watersheds at concentrations that exceeded the Canadian Water Quality Guideline for imidacloprid of 0.23 µg/L. The new generation insecticides, flonicamid and flupyradifurone were also detected in some watersheds, which is the first report of these insecticides occurring in aquatic ecosystems. These data will be discussed in terms of the potential for using POCIS passive samplers for regulatory applications.

**MP218 Calibration of organic-diffusive gradients in thin films (o-DGT) passive samplers for per- and polyfluoroalkyl substances in water**

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Per- and polyfluoroalkyl substances (PFASs) are organic pollutants known to be persistent, toxic and bioaccumulative in the environment, thereby posing a great concern towards human and ecosystem health. In this study, a passive sampling technique that uses organic-diffusive gradients in thin films (o-DGT) was developed for the measurement of six PFASs, including perfluorobutanesulfonic acid (PFBS), perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), and perfluoroundecanoic acid (PFUA) in water. The determination of diffusion coefficients (D) of PFASs at 5 $^{o}$C (1.13–2.46 $\times 10^{-6}$ $cm^2/s$) and 23 $^{o}$C (2.34–3.88 $\times 10^{-6}$ $cm^2/s$) showed that the difference in D values, ranging from 29% to 53%, was a result of slower diffusion at 5 $^{o}$C. The calibration experiment compared measured and predicted sampling rates at 23 $^{o}$C and showed that the average relative error of PFBS, PFOS, PFOA, PFNA, PFDA and PFUA were 3%, 36%, 37%, 37%, 25% and 26%, respectively, which were within 40% of each other, which is reasonable for passive samplers. This observation demonstrated that we can accurately predict in-situ sampling rates using measured D-values, thereby eliminating the need for full-scale calibration. The total uptake capacities of Sepra™ ZT weak-anionic exchange (WAX) binding gels for six PFASs suggested field deployments of 21 days will ensure linear uptake, which are suitable for long-term environmental monitoring, but because of the difference of water pollution at the sampling rates, we suggest that a reduced deployment time e.g.,10 days will also meet the measurement requirements. We conducted field demonstrations of this configuration of the o-DGT in surface waters impacted by PFAS, downstream of two wastewater treatment plants (WWTPs) or industrial areas around Guangzhou, China. The concentration of PFASs ranged from16.9–125 ng/L. This study has demonstrated that o-DGT provides a cost-effective monitoring tool to determine accurate concentrations of PFASs in aquatic systems.

**MP219 Organic-Diffusive Gradients in Thin-Films Passive Samplers: Current State of Research, Applications, and Future Directions**

*J.K. Challis, University of Manitoba / Chemistry; C.S. Wong, University of Winnipeg / Department of Chemistry*

Over the past five years, the organic-diffusive gradients in thin-films (o-DGT) passive sampler has emerged as a promising tool for accurate measurement of polar organic contaminants in aquatic systems. The success of o-DGT stems from the inclusion of an uptake-limiting diffusive hydrogel, greatly reducing the effects of the water boundary layer, and thus in situ flow rate conditions. As a result, o-DGT sampling rates can be estimated based on diffusion through the agarose diffusive gel, facilitating simple and accurate sampler calibration and a means by which to account for temperature effects in situ. A number of studies have successfully tested o-DGT for pharmaceuticals, personal care products, and pesticides, and demonstrated its utility and advantages over current polar passive samplers (e.g., POCIS). However, the o-DGT technique is still new and requires further research to address a number of fundamental questions regarding approaches to sampler calibration, optimized configurations, and mechanisms controlling/impacting uptake. This poster will summarize the current state of this research in the published literature, highlight successful applications, discuss current challenges and knowledge gaps, and make recommendations as to where future research efforts should focus in order to best advance this technique.

**MP220 Toward Toxicological Interpretation of Diffusive Gradients in Thin Films in Marine Waters Impacted by Copper**

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As part of an ambient monitoring program being conducted for the Puget Sound Naval Shipyard & Intermediate Maintenance Facility (PSNS&IMF) in the Puget Sound, receiving waters of Sinclair and Dyes Inlets are routinely monitored for trace metals and toxicity to assess water quality status, track progress in achieving water quality goals, and demonstrate protection of aquatic life. Recently, aqueous metal bioavailability using diffusive gradients in thin film (DGT) passive samplers has been incorporated into the monitoring program. In situ campaigns to record labile ($C_{DGT}$) Cd, Cu, Ni, Pb, and Zn have imbricated DGTs in a manner that allows for response linearity to be defined for deployment times ranging from 24 hours to 14 days in low to moderate ambient conditions, and also in a manner that allows for capture of stormwater related pulses. A current obstacle to regulatory acceptance of $C_{DGT}$ data is validation of an uptake response that reliably mimics that of aquatic organisms. Towards reconciliation of $C_{DGT}$ Cu and natural ligands in respect to toxicity, data shown here correlates ex situ DGT lability of Cu in seawater at varying dissolved organic carbon (DOC) levels to median effective concentration ($EC_{50}$) for *Mytilus* larvae; tantamount to the Marine Biotic Ligand Model. The chronic threshold for $C_{DGT}$ Cu is then applied to gauge the water quality of in situ marine stations monitored over multiple seasons.

**MP221 Development of a novel gas diffusion based passive sampler for monitoring ammonia nitrogen in marine waters**

*L.M. O'Connor Sraj, M.I. Almeida, I.D. McKelvie, S.D. Kolev, The University of Melbourne / School of Chemistry*

Ammonia occurs naturally at low concentrations in aquatic environments, but at elevated levels is highly toxic to aquatic organisms, especially fish. Anthropogenic ammonia pollution of environmental waters has been increasing globally as a result of wastewater plant releases, leaching of ammonia-based fertilizers and agricultural runoff, burning of fossil fuels, and industrial activities. Integrative and passive sampling techniques are an efficient alternative to traditional 'spot' sampling methods, enabling large-scale monitoring programs to assess water quality indicators and the identification of point sources of pollution. Two passive sampling devices (DGT with cation-exchange resin and a polymer inclusion membrane based passive sampler) have been reported for the determination of the time-weighted average concentration ($C_{TWA}$) of ammonium ($NH_4^+$) in freshwaters, but due to interference from alkali and alkaline earth metals in the ion-exchange process neither of these samplers are suitable for monitoring ammonia in marine waters. A passive sampler (PS) based on gas-diffusion across a hydrophobic membrane has been developed and successfully applied for the determination of the $C_{TWA}$ of total ammonia nitrogen in high ionic strength estuarine and coastal waters, for a period of 3 days. This PS is cheap, easy-to-use, reusable, and has a dynamic concentration range of 1.0 – 12 µM ammonia, which covers the water quality guideline trigger value of 11.4 µM (160 µg $L^{-1}$ $NH_3$-N) for high conservation value estuarine and marine waters in Australia. The PS was calibrated under laboratory conditions and deployed in a tank of seawater in the laboratory and at a highly dynamic estuarine site in Port Phillip Bay, SE Australia. Good agreement between passive and spot sampling was achieved in both cases, where the relative error between the concentrations of ammonia nitrogen measured by spot sampling and the corresponding $C_{TWA}$ values was 4.0% for the seawater matrix, and 13.0% for the estuarine site. Membrane permeability and receiving solution optimization studies were performed and the flow pattern effects on ammonia uptake were also examined. Several strategies for extending deployment duration and control of biofouling are under investigation, in addition to a study of the effect of temperature, pH and salinity on the newly developed PS.

**MP222 Testing the effectiveness of stabilized liquid membrane device (SLMD) passive samplers at varying water hardnesses**

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The use of stabilized liquid membrane devices (SLMDs) as freshwater passive sampling devices for ionic metals is an emerging passive sampling technique in environmental monitoring. SLMDs are composed of sealed sections of plastic tubing and filled with a mixture of a chelating agent and oleic acid. The oleic acid interacts with calcium and magnesium ions in the surrounding water to create an outer film in which metals can bind to the chelating agent. Due to ease of construction and low cost, SLMDs have the potential to be useful monitors of metal contamination in freshwater. The samplers have been used in the field under a range of conditions, but have only been laboratory tested in very hard water, which is not representative of all freshwaters that the samplers could be deployed in. It is unknown how SLMD sampling effectiveness changes in soft waters, which may not provide enough calcium and magnesium for the samplers to work properly. To address this knowledge gap, we exposed SLMDs in the laboratory to a fixed concentration of copper in very soft, moderately hard, and very hard water using EPA WET testing recipes. The SLMDs were contained in individual test chambers for 32 days in a static renewal test. Every four days, SLMDs were removed to analyze metal accumulation and remaining test chambers were renewed. Preliminary data suggests that copper accumulation rates are similar in soft and moderately hard waters, but this rate is higher in very hard water. Results will be used to guide future use of SLMDs as passive integrative samplers in water quality monitoring and contaminated site cleanup.

**MP223 Stabilized Liquid Membrane Device Chelation Rates for Metals of Differing Valencies**

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Stabilized liquid membrane devices (SLMDs) are an emerging type of passive sampler used to test for metals in freshwater environments. SLMDs provide an inexpensive alternative to grab samples when testing for metals in freshwater environments. One of the main advantages over grab samples is that SLMDs can represent an average concentration over time, which minimizes the variability that can result from pulses of contamination in freshwater bodies such as streams and rivers. The ability of SLMDs to detect trace metal concentrations that fall below detection limits of most instruments is another advantage over traditional grab sampling. Because of these advantages, SLMDs could have applications in monitoring for contaminated site cleanup. SLMDs contain a 1:1 mixture of oleic acid and Kelex-100, a metal chelating agent. Previous controlled laboratory studies have established the rate of chelation for cadmium, cobalt, copper, nickel, lead, and zinc, all of which are divalent metals. In this experiment, SLMDs were exposed to metals of differing valences including silver (1+), copper (2+), zinc (2+), and aluminum (3+) to investigate potential differences in binding affinity to the Kelex-100. SLMDs were exposed to individual metals as well as mixtures of the four metals. Testing spanned a 32-day exposure period using a static renewal approach. Samples were taken every 4 days to test metal accumulation over time for a total of 8 time intervals. Dilutions were made with moderately hard reconstituted water from EPA Whole Effluent Testing methods. Preliminary results indicate that Kelex-100 has a high affinity for aluminum and zinc relative to copper and silver.

**MP224 A comprehensive in-situ and ex-situ passive sampling program to investigate freely-dissolved metals, inorganic analytes, OCPs, and PCBs**

*M. Healey, J. Roberts, SiREM; J.M. Conder, L. Wellborn, Geosyntec Consultants*

Passive sampling devices (PSDs) present many advantages over conventional sample collection methods for quantifying hydrophobic organic compound (HOC) and inorganic compound availability in sediment, soil, surface water and storm water in terms of cost and data quality. PSDs provide data to estimate contaminant bioavailability and toxicity to environmental receptors that are more accurate than conventional grab or mechanically-extracted samples, as it quantifies freely-dissolved contaminants. PSDs can be deployed directly in situ to capture the effects of groundwater flux, changes in field conditions and the heterogeneity of a site on the freely dissolved concentrations of the compounds of interest. However, physically deploying PSDs can be difficult in the field depending on the foot or boat traffic in the area, the migratory patterns of animals, the depth of the deployment below the sediment or water surface, the current of water above the PSd or even how often a site can be accessed. In these cases where in situ deployment is not feasible, samples can be collected once from the field and analyzed using ex situ methods. In addition to the above advantages, ex situ experiments can be mixed to decrease deployment times, allowing kinetically slow compounds to be analyzed under shorter time frames and the results of the PSD can be directly compared to the sediment it was deployed in. Ex situ experiments, however, remove the PSD from true field conditions. In this experiment, PSD peepers for metals, ammonia, fluoride analysis were deployed under in situ conditions to surface sediment and deeper sediment samples from an undisclosed site to determine the risk associated with these compounds at the site. Ex situ testing was additionally completed for organochlorine pesticides and polychlorinated biphenyl (PCB) analysis using a polyethylene-based PSD with rare PCB congener performance reference compounds. Discussion comparing the in situ and ex situ deployment will be explored as well as a comparison of the results between the two deployment methods.

**MP225 The development of a passive sampling device to evaluate dissolved oxygen concentration in benthic zone**

*Y. Kameda, Chiba Institute of Technology / Creative Engineering*

Monitoring of the concentration of dissolved oxygen is very important to keep a broad spectrum of benthic organisms, healthy ecosystem and good cycle of nutrients and pollutants in lakes and the sea. In Japan, dissolved oxygen is one of water quality standards in lakes and bays. However, it is very hard work to monitor the DO concentration frequently at many sampling sites and evaluate the spatial distribution. DO sensors are very useful but very expensive. The grab sampling method is less expensive than monitoring by sensors but the data is snapshot data. The DO monitoring will need continuous data for benthic organisms because the aquatic environment in benthic zone is heterogeneous spatiotemporally. This presentation shows a novel monitoring technique to measure DO concentration in benthic zone in lakes and the sea by using a novel DO passive sampler. The passive sampler consists of chemcatcher holder, oxygen absorbent film and diffusion limiting rubber. The color of the film changes according to the absorbed amount of DO. Therefore the absorbed amount can be estimated from the color. The color can be qualified and quantified by low cost color difference meters. Laboratory calibration tests revealed that the absorbed amount of DO increased linearly during the deployment. The calibration tests also revealed sampling rates and effects of flow rate and water temperature to them. After that, this device including a special cage will be applied to lakes and the sea to compare time weighted average concentration of DO by passive samplers with those from continuous monitoring data by using DO sensors. This device is very easy to operate and low cost so that it will be one of useful techniques to monitor spatial distributions and temporal variation of DO concentration in benthic zone in lakes, bays and the sea.

**MP226 Application of Passive Sampling to Measure PCB Microbial Dechlorination Kinetics**

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Progress in developing suitable microbial bioremediation treatments of polychlorinated biphenyl (PCB) in contaminated sediments have traditionally struggled to measure and model the microbial kinetics at environmentally relevant concentrations. To address this issue, we used low-density polyethylene (PE) for passive dosing/sampling and quantified the biological rate of dechlorination of 2,3,4,5-tetrachlorobiphenyl (PCB 61) to 2,3,4-trichlorobiphenyl (PCB 23) by the organohalide respiring bacterium, *Dehalobium chlorocoercia* (DF-1). The biological rates were measured over environmentally relevant concentration ranges of 1.5-40 ng/L aqueous concentration with and without the presence of sediment in bench scale microcosm studies. The rate of dechlorination was determined to be linearly dependent on the initial freely dissolved concentration of PCB 61 both in sediment and sediment free microcosms. Predicted rates of dechlorination based on sediment free microcosms were within a factor of 2 in sediment microcosms. Passive dosing/sampling approach reported in this study, combined with partitioning characteristics of specific sediments, can be used to predict in-situ rates of microbial dechlorination during bioaugmentation or monitored natural attenuation to enable future microbial bioremediation treatment options.

**MP227 Ex Situ Chemical Availability Recontamination Grab Observation (ESCARGO) For Rapid Assessment of Sediment Amendments**

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Approaches to quantify the reduction in chemical availability in sediments amended with solid materials (e.g., sand, dredged material (DM), activated carbon, etc.) can involve complicated, expensive, and time-consuming bench- or pilot-scale evaluations. These evaluations often do not (or cannot) evaluate the long-term conditions in which the solid amendment has been fully or partially mixed with underlying contaminated sediment through bioturbation or other natural processes. In response to these challenges, we devised and tested a rapid method to assess the long-term reduction of chemical availability gained from solid amendments: the *Ex Situ* Chemical Availability Recontamination Grab Observation (ESCARGO). The ESCARGO approach combines a small sample of unamended site sediment (~10-50 g, dry weight), the sediment amendment(s) of interest, and a polyethylene (PE) passive sampler under agitated conditions for 28 days. Following the equilibration period, the PE is analyzed to measure concentrations of freely-dissolved organic chemicals. Three treatments are assessed: 1) an unamended sediment; 2) a 10% sediment/90% amendment mixture to represent "short term" remedy performance; and 3) a 40% sediment/60% amendment mixture to represent "long term" remedy performance. The ESCARGO approach was applied to evaluate the reduction in PCB availability for a sediment in Pearl Harbor amended with 3 amendments (sand, and two DMs with 0.44% and 0.99% Total Organic Carbon (TOC) contents). ESCARGO short term results indicated that the higher carbon DM reduced available PCBs by an average (SD) of 81% (± 5%), performing better than the lower carbon DM and sand (reduction in PCB availabilities of 51% (± 6%) and 45% (± 9%), respectively). ESCARGO long-term results also indicated superior performance of the higher carbon DM, but indicated slightly lower reductions in PCB availability, as compared to the short term results (reduction in PCB availabilities of 66%, 39%, and 37% for higher carbon DM, lower carbon DM, and sand, respectively). Results suggest additional PCB binding capacity of the higher carbon DM compared to the sand and lower carbon DM, and project long-term reduction in PCB availability. The ESCARGO approach is a rapid and inexpensive method to compare the site-specific performance of multiple contaminated sediment amendments and provide a prediction of their long-term performance.

**MP228 Use of ex situ passive samplers to measure freely dissolved PAHs in sediments at a former manufactured gas plant site**

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Concentrations of freely dissolved polycyclic aromatic hydrocarbons (PAHs) in sediment porewater have been shown to be related to the bioavailability of sediment-associated PAHs to aquatic organisms. The objective of this work was to demonstrate the use of passive samplers in the laboratory (*ex situ*) for measuring the concentration of freely dissolved PAHs in porewater from impacted sediment at a former manufactured gas plant site. Nine samples of surface sediment were each homogenized and split for 1) analysis of bulk sediment for 34 PAHs, total organic carbon, and black carbon and 2) exposure to polyethylene (PE) passive samplers in the laboratory. Pre-cleaned PE strips were added to sediment slurries in 500-mL glass jars and placed on a shaker table for six weeks. At the end of the sixth week, PE strips were retrieved, cleaned, and extracted immediately. Freely dissolved PAH concentrations in porewater were calculated from concentrations in PE using PE-water partition coefficients and compared to porewater concentrations predicted from equilibrium partitioning models. Results indicate that concentrations measured using passive samplers are lower than concentrations estimated using standard sediment organic carbon to porewater partition coefficients ($K_{oc}$), but higher than concentrations estimated using a partitioning model that incorporates sorption to both natural sediment organic carbon and black carbon. The Sum of Toxic Units (Sum-TU) based on concentrations of 34 PAHs in bulk sediment >1 indicates further assessment is warranted for these sample locations, but the Sum-TU based on concentrations of PAHs measured using passive samplers < 1 indicates concentrations of PAHs in these samples are acceptable for the protection of benthic invertebrates. These results indicate that *ex situ* passive samplers 1) can be used to measure concentrations of freely dissolved PAHs in porewater and 2) may provide a more accurate measurement of concentrations of bioavailable contaminants, which can be used to “screen out” samples or areas of a site that require no further analysis of risk.

**MP229 Performance Evaluation of an Engineered Sediment Cap for Chemical Isolation Using In Situ SPME Passive Samplers**

*M. Kanematsu, D.R. Opdyke, D. Vlassopoulos, K. Russell, Anchor QEA*

An engineered sediment cap was installed after dredging 10 feet of glacial till contaminated by polychlorinated biphenyls (PCBs) to prevent leaching of remaining PCBs from underlying material in the River Raisin. The cap consists of a 1-foot chemical containment layer (CCL) comprising sand and organoclay to isolate PCBs, overlain by a gravel filter layer topped with armor stone. Following cap placement, porewater monitoring was performed to verify the integrity and effectiveness of the CCL. For porewater monitoring, three sand-filled monitoring ports were installed at different locations in the cap through the filter and armor layers. One year after placement, in situ solid-phase microextraction (SPME) passive samplers were deployed in the monitoring ports to measure PCB concentrations in the porewater just above the CCL. Each SPME sampler consisted of a 5-foot-long custom-designed push-point sampler holding two SPME fibers (4 inches long with a 35-micron-thick polydimethylsiloxane coating) and was designed to allow porewater to contact the SPME fibers while also preventing vertical preferential flow paths inside the sampler. Because highly hydrophobic PCB congeners may require months or even years to reach equilibrium between the fibers and surrounding porewater, the SPME fibers were spiked with six $^{13}C$-labeled PCBs prior to deployment as performance reference compounds (PRCs) to provide information about site-specific mass transfer kinetics of PCBs. At the end of the deployment period (105 days), the SPME samplers were successfully retrieved without damage. An external resistance model, based on Lampert et al. (2015) and Fernandez et al. (2009), was fit to the PRC data to estimate site-specific mass transfer rates for all measured PCB congeners. The estimated total PCB (TPCB) porewater concentrations in the three monitoring ports were similar to the TPCB concentrations in the overlying water column (measured using semi-permeable membrane devices), which is consistent with expectations, given that surface water was entrained in the monitoring ports during sand placement. The porewater TPCB concentrations were substantially lower than the TPCB concentrations in the pre-construction sediment porewater. These results demonstrate that the CCL is performing as designed and limiting migration of PCBs to the water column. Installation of the monitoring ports and custom-designed SPME samplers allowed successful porewater sampling in the sediment cap.

**MP230 Application of passive sampling for quantification of sources and sinks of PCBs and OCPs in the Anacostia River**

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Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are major contaminants of concern in the Anacostia River, resulting in fish-consumption advisories in District of Columbia (DC). To quantify the sources and sinks for these pollutants, polyethylene (PE) passive samplers were deployed at several locations in DC to measure the freely dissolved surface water and sediment porewater concentrations of OCPs and PCBs. Passive samplers were also concurrently deployed at 5 locations in Washington DC to measure the gas-phase concentrations. Our initial results show that the freely-dissolved PCB concentrations in the water column across the sites varied from 0.01 – 6.5 ng/L while measured OCP concentrations were in the range of 0.22 – 8.4 ng/L during the deployment period from March to July 2017. During the same period, gas phase PCB concentrations ranged from 208 – 1015 pg/m$^3$, while gas-phase OCP concentrations were in the range of 80 – 122 pg/m$^3$ across the sites. The water-air exchange flux for PCBs and OCPs over the Anacostia River was calculated to be +282 ng/m$^2$/day and +207 ng/m$^2$/day respectively, resulting in volatilization of about 355 g of PCBs and 260 g of OCPs per year from the Anacostia river. Measurements of dissolved and particulate organic carbon (DOC and POC, respectively) and direct measurements of PCB and OCP concentrations on suspended sediments are being used in combination with streamflow data from United States Geological Survey (USGS) to calculate incoming pollutant loads from major tributaries and branches of the Anacostia river and the net pollutant outflow from the Anacostia into the Chesapeake Bay. The pollutant transfer rates between sediments and overlying water due to diffusive flux are also being quantified. Data analyses are ongoing for samples taken through a full one-year cycle to quantify seasonal differences that will allow a complete understanding of the annual pollution budgets for the Anacostia River.

**MP231 Evaporative Emissions of Polycyclic Aromatic Compounds from Alberta’s Oil Sands Tailings Ponds Water**

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Polycyclic aromatic compounds (PACs) are semi volatile organic compounds and emitted from Alberta’s oil sands tailing ponds (OSTPs). These compounds are important because they are known to pose health hazards and be atmospherically transported to non-source regions. Water samples

taken from the ponds in the summers of 2016 and 2017 were analyzed for PAH and alkylated-PAHs, additionally they were screened for nitro and oxy-PAHs. Preliminary results indicate the levels of alkylated-PAHs are higher than PAHs by about an order of magnitude. . These open-aired ponds are shallow and have a large surface areas, thus organic compounds can easily volatize into the atmosphere. Evaporative emissions of PACs from these ponds are estimated using air-water partition coefficient values determined under ideal conditions (i.e., de-ionized water) and may have associated artifacts from the methodology. OSTP water is a complex mixture containing compounds that might affect these partitioning values. So an equilibrium chamber study was devised to more appropriately determine the air-OSTP water partition coefficients for PACs. This chamber study setup used ethylene vinyl acetate (EVA) polymer as a thin-film passive sampler for fugacity measurements of PACs in air and water. This same experiment was successful using deionized water for the distribution of PAHs between air and water. We will present the levels of PACs in OSTP waters, the results of the chamber experiment using the complex mixture of OSTP waters and estimate the net emissions of PACs from OSTPs using paired water and passive air samplers.

**MP232 Assessing the atmosphere-surface exchange of gaseous elemental mercury using passive air samplers**

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The specific properties of gaseous elemental mercury (GEM) allow it to undergo bidirectional exchange between the atmosphere and the Earth's surface. Determining the direction and the magnitude of GEM's atmosphere-surface flux is possible and has been accomplished using micrometeorological and chamber techniques, but is (i) complex and labor intensive, and (ii) often only yields fluxes over relatively short time scales. A recently developed passive air sampler for GEM may have the precision required for identifying and quantifying vertical concentration gradients above the Earth's surface and therefore the direction and size of exchange fluxes at much longer time scales of weeks to a year and with much less effort and lower cost than existing approaches. We tested the feasibility and performance of this approach by measuring concentration gradients for one year with a monthly resolution above both natural soil in a forest and a nearby clearing, as well as above soil artificially contaminated with inorganic mercury. Significant gradients of GEM air concentrations, both increasing and decreasing with height above ground, were observed and found to be dependent on soil contamination, temperature, snow cover and solar irradiation. Because the sampler's uptake kinetics has a known, small dependence on wind speed, sampled amounts of Hg that decrease with increasing proximity to the soil surface could be an artifact caused by wind speed gradients. Measurements, however, reveal that wind differences above the soil are insufficient to explain the differences in the sampled amounts with height, implying that deposition of GEM can be recorded with this method. The method holds promise to also find use for the measurement of GEM fluxes between atmosphere and vegetation, e.g. in forest and tundra ecosystems.

**MP233 Development of guidelines for use of passive sampling devices for natural resource damage assessment and restoration**

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Passive sampling devices (PSDs) are an effective, fit-for-purpose technology that offer an alternative to conventional sampling techniques for characterizing bioavailable contaminant concentrations in aquatic environments. Recent improvements and validation of methods to quantify aqueous concentrations from PSDs, especially for nonpolar PSDs, as well as additional standardization and validation of passive sampling methods have transformed PSDs into a robust, quantitative technique. While PSDs have the potential to become an integral part of the National Oceanic and Atmospheric Administration Assessment and Restoration Division's (NOAA/ARD's) "toolkit" for conducting natural resource damage assessments (NRDAs) at waste sites and oil spills, general unfamiliarity with the methods and lack of specific guidelines have prevented widespread adoption. The main objective of this study is to develop guidelines on the use of PSDs in NRDA to enable the uptake of this technology within ARD. The aim is to help ARD project managers determine when PSDs may be useful during an assessment or restoration monitoring and if useful, to provide the resources and information needed to help implement passive sampling successfully at NRDA waste and oil spill sites. In determining if PSDs are suitable for a particular site characterization or exposure or injury assessment, we discuss common NRDA data quality objectives and how PSDs could be used to address them. For these initial guidelines, we chose to focus on low density polyethylene (LDPE) membrane PSDs since they are well-established, readily available, easy to use, low cost, and are supported by commercial analytical laboratories. For LDPE PSDs, we describe the methods for preparing and analyzing PSD samples, as well as identify several commercial laboratories that can help with these steps. We also provide protocols for deploying and retrieving PSDs in the field, presenting examples of different sampler and deployment configurations. We discuss deployment timing and sampling design, especially as it relates to different waste site and spill scenarios where contaminant concentrations may be changing with time. Finally, we provide procedures needed to process and interpret the data. These guidelines aim to bridge the gap between research and application by end-users in the context of the specific needs and objectives of NRDA.

**MP235 Modeling the impacts from non-linear sorption to the behaviors of PRCs and target compounds in passive sampling**

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In the past decade, passive sampling with organic polymers has been used for the assessment of bioavailability of hydrophobic organic contaminants in sediment. Performance reference compounds (PRC) have been considered as a reliable calibrating approach for estimating uptake rates of the target contaminant in non-equilibrium passive sampling. The traditional use of PRCs is to assume that the sorption of a target compound and desorption of its corresponding PRC follow the same rate. With nonlinear sorbents such as activated carbon mixed into sediments, PRCs do not exhibit desorption rates equivalent to similar compound uptake rates and the difference is a function of compound concentration and the nonlinearity of the sorbent sorption. In this presentation, a one-dimensional diffusion model with non-linear sorption has been developed to study the impacts from the non-linear sorption to the asymmetric behaviors of PRCs and target compounds. The numerical simulation of the model is performed by CapSim – a robust numerical modeling tool, and the simulated results are compared both qualitatively and quantitatively to experimental data from recent studies that observed the asymmetric behaviors. The presentation provides estimates of the errors in assuming reversible sorption and desorption and how to limit those area through property control of PRC loading of the passive sampler. When PRC loading cannot be controlled, a methodology for correction will be presented. The results will be applied to measure porewater concentrations of PCBs at several sites where activated carbon has been used to manage bioavailability.

**MP236 Evaluating partition-based sampling for predicting the bioaccumulation of hydrophobic organic contaminants by higher trophic level organisms**

*S.N. Schmidt, Technical University of Denmark / Atlantic Ecology Division; R.M. Burgess, USEPA / Atlantic Ecology Division*

Recent research has shown that bioaccumulation of hydrophobic organic compounds by benthic and sessile marine organisms can be accurately predicted by partitioning-based sampling (within a factor of 10). In these lower trophic level organisms, bioaccumulation can thus be explained by equilibrium partitioning. Now, research is needed to assess the ability of partitioning-based sampling to estimate bioaccumulation by pelagic and mobile organisms (i.e., by higher trophic level organisms). The limited available research on the topic states that processes beyond simple equilibrium partitioning need to be taken into consideration to accurately estimate bioaccumulation by these organisms. Generally, two partitioning-based (passive) sampling approaches are used: (1) the *ex situ* equilibrium sampling approach fully equilibrates a thin polymer with sediment during tumbling in the laboratory and (2) the in situ pre-equilibrium sampling approach places a thicker polymer within the bulk water or sediment on site and then infers equilibrium concentrations through the use of performance reference compounds (PRCs). As noted above, the equilibrium polymer concentrations can be used as a surrogate predictor of bioaccumulation in lower trophic level organisms. In addition, freely dissolved concentrations ($C_{free}$) can be determined as the ratio of the equilibrium concentrations in the polymer and polymer to water partition ratios. The current study reviews existing literature linking partitioning-based sampling data and bioaccumulation by higher trophic level shellfish and fish. The equilibrium polymer concentrations and $C_{free}$ determined by partitioning-based sampling are incorporated into bioaccumulation models to predict wet weight concentrations, lipid-normalized concentrations and chemical activity in higher trophic level organisms. Ideally, this research will provide an assessment of the ability to predict bioaccumulation by higher trophic level organisms using partitioning-based sampling approaches. If successful, this research will serve as a tool for regulatory agencies (e.g., USEPA) to assess risk associated with contaminated sediments in terms of their potential to contaminate commercially and recreationally important shellfish and fish.

## Advances in Sediment Quality Assessment for Regulation and Management

### MP237 An Absorption Sink Coupled with Isotope Dilution Method for Improved Assessment of Bioavailability of Organic Pollutants

*X. Li, University of California, Riverside; J. Gan, University of California, Riverside / Environmental Science*

The use of bulk chemical concentration for environmental risk assessment of hydrophobic organic contaminants (HOCs) in soil or sediment is considered to overexpress their ecotoxicity, as HOCs have high affinity for solid particles and irreversible sorption. The measurement of bioavailability of HOCs is important for improving their risk assessment. Biological methods such as bioaccumulation offer direct measurement of bioavailability, but they are often expensive and extremely time-consuming. Several chemically based methods for assessing the bioaccessibility of HOCs have been developed. Isotope dilution method (IDM) is a relatively new method, which makes the use of the identical properties and behaviors of target analytes and their isotope labeled counterparts. However, in the conventional protocol of the IDM method, usually only a subsample of the aqueous phase was used for analysis to derive the ratio of the target analytes and their isotope labeled analogues following GC-MS analysis. For strongly hydrophobic compounds, this method often lacks sensitivity and reproducibility because the analytes are predominantly in the solid phase. In the current study, we introduced an absorption sink (e.g., silicone rod, polyethylene film) in the IDM method to increase the quantity of target and isotope labeled analytes for analysis. After mixing, the absorption sink was removed and extracted with an organic solvent before analysis. This simple modification showed a significant improvement in the sensitivity and reproducibility of IDM for a range of HOCs including DDTs and PCBs in soils and sediments.

### MP238 Removing Tenax system dependence during contaminant risk evaluation through phase volume normalization

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Tenax extractions estimate exposure risk of sediments contaminated with hydrophobic organic compounds (HOCs) by measuring the chemical fraction that is and will become available for exposure. Normalizing Tenax extractable concentrations for sediment organic carbon (OC) corrects extractable concentrations for the environmental fraction controlling bioaccessibility, allowing for rapid screening of bioaccumulation risk. This normalization, however, encumbers this technique with a system dependence absent from similar chemical extraction methodologies, such as passive samplers. Passive sampler concentrations are normalized for the phase volume of the sorbent coating the device, such that representing chemical to sampler partitioning is independent of the extraction system. As such, implementation of this methodology in risk assessment is straightforward. Normalizing Tenax extractable concentrations for the Tenax phase volume similarly to passive samplers would remove the Tenax system dependence, extending applicability in environmental risk assessment. The objective of the current study was to mathematically determine the Tenax phase volume, and compare Tenax phase volume normalization to OC normalization in relation to estimating bioaccumulation using single-point Tenax extractions. Based on the surface area of 35 $m^2$ for 1 g of Tenax, the calculated Tenax phase volume was equal to 5.9 L/g Tenax. Normalizing Tenax extractable concentrations from previous studies for the Tenax phase volume used during single-point Tenax extractions and comparing these values to bioaccumulated tissue concentrations of aquatic invertebrates yielded linear regressions similar to those generated with OC normalization. The correlation coefficients, parameters of the regression lines, and statistical significance of the regressions did not change when normalizing Tenax concentrations for phase volume or sediment OC within studies. Combining data from the previous studies in a single regression did not significantly affect the estimates of bioaccumulation based on Tenax extractable concentrations normalized for phase volume or OC, meaning Tenax phase volume normalization comparably corrects Tenax extractable concentrations to OC normalization. Without measuring OC content of sediment prior to estimating HOC contamination risk, Tenax offers a rapid, cost-effective, and reliable means of screening contaminated sites during environmental risk assessments.

### MP239 Influence of Complex interactions between Tubificid Oligochaetes and Bivalves on Metal Bioavailability in Multi-metal Contaminated Sediments

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Biological activities by benthic organisms perturb the physical, chemical and biological structures of sediments, modifying the fate of metal contaminants within and the release from sediments. Quantifying the influence of bioturbation on contaminant bioavailability is important for assessment of sediment quality and associated contamination risk. In the present study, we studied the effects of bioturbation on efflux of metals from multi-metal contaminated sediments, with particular emphasis on the interplay between organisms belonging to different functional groups. Multi-metal contaminated sediments with in situ living oligochaetes *Limnodrilus hoffmeisteri* were collected from the field, incubated and stabilized under the laboratory condition for three months to reconstruct a steady biogeochemical structure within the sediment. The bivalves, *Corbicula fluminea*, were then introduced into the mesocosm at different densities. Metal concentrations in the overlying water were monitored daily for a period of 8 days. Diffusive gradients in thin film (DGT) probes were deployed at the end of the experiment to determine the in situ porewater metal concentrations. Total dissolved metal fluxes were determined based on metal concentrations in the overlying water and the diffusive fluxes were derived based on the porewater profiles. The results indicated that the interplay between the two groups of organisms enhanced the release of Mn, Co, Ni and Zn. Efflux of Mn, Co, Ni and Zn were also significantly elevated, with bioturbation induced efflux significantly higher than the diffusive efflux. However, the mobilization and efflux of Cu and Cr were not significantly different between treatments and was attributed to the higher affinities of these two metals to the solid phase resulting in lower mobility of Cu and Cr. Overall, our study demonstrated that the responses of different metals to the same bioturbation behavior was different, resulting in distinct mobility and fate of metals in the sediment, and modifying the bioavailability to the main bioturbating organisms and cohabiting organisms.

**MP240 Effects of physico-chemical characteristics of formulated sediment on the survival and growth of amphipods *Grandidierella japonica* and *Hyalella azteca***

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Spiked sediment toxicity tests are necessary for the hazard/risk assessments of hydrophobic chemicals or substances strongly binding to sediment. In the spiked sediment tests, the use of formulated sediment is occasionally preferred rather than field-collected sediment because of its higher reproducibility and availability regardless of seasons and places. Although several types of formulated sediment have been proposed, little is known about the effects of physico-chemical characteristics of formulated sediment (e.g., particle size distribution and types of organic matter) on amphipods and thus it is still unclear about what is the best formulated sediment. In this study, we investigated the applicability of several types of formulated sediment using a freshwater amphipod *Hyalella azteca* and an estuarine amphipod *Grandidierella japonica*. As a preliminary experiment to investigate the effects of particle size distribution on amphipods, we performed 10-day survival and growth tests of formulated sediment comprising of quartz sand and kaolin using *G. japonica*, with changing the proportions of kaolin as silt and clay particles (0, 5, 10, 25, 50, and 100%). Our experiments demonstrated that while all formulated sediment sample was not lethal toxic (mortality < 10%) to *G. japonica*, the body length increment of surviving amphipods was significantly different among the proportions of kaolin. The increment was the highest in the sediment comprising of 5% to 10% kaolin, and was decreased with increasing kaolin proportions. Based on these results, we hypothesized that standardized formulated sediment containing large proportions of silt and clay (about 20%) is not always appropriate for amphipod toxicity tests. To validate the hypothesis, we conducted the same experiments using *H. azteca*. In addition, we will present the results comparing several standardized formulated sediment samples (e.g., ISO, USEPA, and OECD) using survival and growth tests of *H. azteca*, and investigate the effects of differences in organic matter (e.g., peat moss and α-cellulose) and the other ingredients on the amphipod.

**MP241 Sediment Toxicity Testing at a Reduced Temperature: Diagnostic Results from California's Stream Pollution Trends (SPoT) Monitoring Program**

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The Stream Pollution Trends Program (SPoT) is a statewide sediment toxicity and contaminant monitoring program that is part of California's Surface Water Ambient Monitoring Program (SWAMP). Since 2008, SPoT has monitored integrator sites in up to 100 watersheds for toxicity to the amphipod *Hyalella azteca* and a number of current-use and legacy contaminants. Amphipod toxicity has remained consistent with approximately one in five monitored sites significantly toxic every year. The observed toxicity is significantly related to urban land use and concentrations of pyrethroid pesticides. The relationship between toxicity and pyrethroid pesticides was confirmed, in part, by synoptically testing a subset of samples at two different temperatures. Pyrethroids become more toxic as temperature decreases, so tests were conducted at the standard temperature of 23°C, as well as at 15°C. This lower temperature better represents the average temperature for surface waters at SWAMP sites between 2001 and 2010, which was 15.8°C. Between 2010 and 2016, 194 samples were tested at both temperatures. Significant toxicity was observed in 21% of the samples tested at 23°C, whereas 59% of the samples were significantly toxic at 15°C. Samples containing greater than five organic carbon-corrected toxic units of total pyrethroids were generally toxic to *H. azteca*. Testing at 15°C shifts the toxicity threshold to a lower value. This data set demonstrates the utility of this pyrethroid toxicity screening tool on a large scale. These data also suggest that the potential for surface water toxicity is likely underestimated in SPoT watersheds based on assessing toxicity at the standard protocol temperature.

**MP242 Evaluation of sediment toxicities in the Ariake Sea, Japan, using marine medaka embryos**

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Chemicals derived from human activities have been carried to the sea area by river water, rainfall, and other factors, and then are settled on sediments finally. They could have individual or multiple effects to the benthic animals, but their actual effects are almost unknown. Recently, we have been developing new method for the evaluation of sediment toxicities collected from the field, using embryos of Java medaka (*Oryzias javanicus*) as marine fish. Because ithe embryos are kept on the polluted sediment only with slight pore water and without overlying water, this can evaluate the toxicities in sediment alone. The Ariake Sea is located in western part of Kyushu, Japan, and has huge area of tidal flats. Although the abundance of aquatic organisms, such as mudskipper fish, crabs, clams, and others, inhibits there, they has dramatically decreased in recent years. Some researchers reported the chemical distributions in sediments of the Ariake Sea, for example, persistent organic pollutants as polychlorinated biphenyls, endocrine disrupter chemicals, and others. However, there have been no examinations on the sediment toxicities to aquatic organisms. In the present study, we collected the field sediments at 12 locations along the shoreline of the Ariake Sea in December 2016. and evaluateed sediment toxicities using the embryos of Java medaka The dried sediment samples were rinsed with an embryo raring mediums. Ten embryos were embedded in half of them in sediment, which was laid at a glass petri dish with 35 mm of diameter, and were developed on the sediment for 12 days post-fertilization. In the period, the rearing water was not added to the dishes. Three dishes contained the sediment were prepared as sampling site (n=3). An embryo was then individually transferred in a hole of 48-well microplates containing artificial sea water, and kept until 3 days after hatching. In this evaluation, sediments collected at 2 locations (mouth of Shiota River) in northeast of the sea were killed 96.7% of embryos. Additionally, the mortality rates of 40 – 77 % were observed in sediments collected from the mouths of Kise, Hattae, and Chikugo Rivers. These perhaps resulted that some toxicants drifting with river flowing and settling on sediments of the river mouth affected to embryos. Our evaluations suggest that sediments at some locations of the Ariake Bay seriously affect to aquatic organisms.

**MP243 Organophosphate esters in the marine sediments of the Palos Verdes Shelf: Spatial and temporal trends**

*J. Li, J. Gan, University of California, Riverside / Environmental Science*

Organophosphate esters (OPEs) are an important class of flame retardants that have extensively used for recent decades. In this study, we measured the concentrations of 10 OPEs in surficial sediments collected from 44 locations at the Palos Verdes Shelf Superfund site off the coast of Los Angeles, and three sedimentary cores were used to understand the temporal trend of their contamination. In the surficial sediments, OPE concentrations ranged from 2.03 to 277 ng/g, and exhibited a logarithmical decrease with relation to the distance from the effluent outfall of wastewater treat plant (WWTP). The strongly hydrophobic OPEs tended to accumulate in offshore areas, whereas the less hydrophobic OPEs appeared to accumulate in inshore areas. The ratios of OPE concentrations between offshore and inshore sites were negatively related to their log Kow values. The sediment cores were dated using $^{210}Pb$, and levels of OPEs in the sediment initially increased during 1940-1970s, and then subsequently decreased in the 1980s. This variation coincided with the historical emission of effluent and suspended solids from WWTPs. After the 1990s, another increase in OPE concentrations was observed and their increase was more pronounced compared to PBDEs. This finding likely reflected the increasing demand for OPEs as the replacement for legacy flame retardants such as PBDEs in the U.S. The ratios of metabolites/parent compounds, including BBOEP/TBOEP and BDCIPP/TDCIPP, were calculated. The ratios showed active in situ natural transformations. This study showed that WWTPs and other urban emissions of OPEs have resulted in the accumulation of OPEs in the marine environment, from where these persistent contaminant may participate in the global ocean cycle.

### MP244 Weight of Evidence Approach for Benthic Metals Assessment

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At sediment sites with metals contamination, data collected for the purpose of evaluating benthic organism exposure to metals (i.e., exposure data) may include bulk sediment chemistry, excess simultaneously extracted metals (SEM) in sediment, and dissolved metals concentrations in porewater. Also for use at such sites, multiple approaches are available to assess toxicity, such as sediment quality guidelines (SQGs), excess SEM, and other types of equilibrium partitioning sediment benchmarks (ESBs) for sediment-related exposures, including ambient water quality criteria (AWQC), bioavailability-based regression models, and porewater biotic ligand models (BLMs). When the different exposure data are linked with the different toxicity assessment approaches, the result is multiple lines of evidence (LOEs) that can be used to evaluate hazards from metals in sediment and porewater at a particular location. We applied the US Environmental Protection Agency's (EPA's) 2016 *Weight of Evidence in Ecological Assessment* framework to weigh multiple LOEs for evaluating hazards to benthos from metals. Assuming all sediment and porewater data were of acceptable quality, the relevance, strength, and reliability of each LOE was evaluated—including evidence describing expected chemical interactions in a mixtures-toxicity framework—and assigned a qualitative score. Evidence considering bioavailability received higher relevance and strength scores because the relevant form was considered and association with the causative agent was high; evidence considering mixture effects received higher relevance and reliability scores because this evidence better reflects environmental exposures and is consistent with understanding of common mechanisms of metals toxicity. Evidence relying on correlative, rather than causative, toxicity information received lower strength and reliability scores. We found that EPA's weight of evidence (WOE) framework facilitates the logical synthesis of evidence to support conclusions. This WOE approach for the assessment of hazards to benthos from metals will prove useful in understanding the limitations of conclusions drawn from weaker evidence and in prioritizing efforts to obtain additional information (e.g., bioassays, ecological community data, etc.)

### MP245 Site-Specific Sediment Criteria: A framework for using bioassays as a tool for risk assessments

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As part of the risk assessment framework, sediment constituent loads are often compared to common, but loosely relevant, benchmark levels as part of determining the potential for an impact to biological life. There are several technical and regulatory scenarios that benefit from the development of site-specific sediment criteria, including constituent- or condition-specific information gaps or the lack of observed biological response in previous monitoring. Bioassays have the ability to serve as a powerful, integrated tool to support a decision maker's weight-of-evidence approach. The participation of scientists with expertise in bioassay methods and their proper adaptation and interpretation are invaluable to a risk assessment team as they develop goals and plan sampling and analysis design. Site-specific criteria can be calculated using sediment amendment (i.e., spiking studies) and subsequent biological evaluation to more fully characterize local conditions as well as the viability of potential remediation alternatives. When undertaking these studies, multiple factors critical to understanding the design should be considered during planning stages, well before execution of any field work. This presentation aims to provide context, applications, and considerations for bioassay testing as it relates to risk assessment beyond a typical monitoring regime. A case study will form the basis for highlighting key elements and reviewing considerations and variables that should be addressed to ensure a positive outcome and successful regulatory participation and acceptance. We will break apart and explain aspects and elements of the design, small and large, which can be the difference that leads to successful execution and acceptance of the data. From a regulatory standpoint, clear study design and early, frequent communication are paramount to gaining acceptance of both the assessment and subsequent implementation plans. Test design should contemplate a number of physical and chemical elements which include but are not limited to: formulation/speciation of the target compound, parameters for sediment amendment, media for amendment, fidelity of site water/sediment chemical parameters, and method modifications to address site-specific conditions. The ability to understand and communicate the importance of these factors during the planning stage increases the likelihood of acceptance for the proposed plan and, later, results and recommendations.

### MP246 Evaluating Risk in Freshwater Mining Sediments of Tasmania

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Mining-impacted aquatic systems could be at risk from various pollutants, including heavy metals, sulfate, and acidic pH. The present study first evaluated toxicity of stream sediments downstream of metal mines in western Tasmania using a conventional contaminant-based approach (i.e. comparing chemical concentrations to published literature and then comparing this observations to observed toxicity). In this study, we used two native Australian species, *Chironomus tepperi* (midge) and *Austrochiltonia subtenius* (amphipod), to evaluate toxicity of site sediments. Results from these bioassays showed that many of the aquatic systems in Western Tasmania were negatively impacted by current and historic mining activities. Interestingly, the two species showed differing toxicity responses; which may have been the result of different species sensitivities or experimental conditions—suggesting that methods and species sensitivity would need to be considered carefully in future risk evaluations in this area. These sediments were also chemically evaluated for a suite of metals as well as sulfate. This evaluation, coupled with the observed pH in bioassays, provided only a limited understanding of causality (as all three contaminants appeared to be causing risk). As such, we attempted to better understand risk using modified whole-sediment TIE techniques, wherein a cationic resin (to characterize metals toxicity) and an anionic resin (to characterize sulfate and acidic pH toxicity) could be used to better understand causality. Unfortunately, the complex and linked nature of the pollutants (namely acidity driving metals toxicity) in these site sediments meant even this technique could not resolve the activities of the respective pollutants, and overall did not elucidate the causal nature of toxicity. The current presentation will outline the pitfalls of using the conventional approach as well as the strengths, current limitations, and future directions of using TIE techniques for mining sediments.

## Predictive and Statistical Toxicology

**MP247 Assessing the effects of silver nanoparticles on ARPE-19 cells via high content imaging-based phenotypic profiling**

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Efficient methods are needed to evaluate the human and environmental health effects of silver nanoparticles (AgNPs). A high content imaging-based phenotypic profiling approach was used to determine the effects of 12 types (4 sizes x 3 coatings) of AgNPs on organelle morphology in human retinal pigmented epithelial (ARPE-19) cells. AgNPs (0.1-30 µg/ml) were applied to the cells seeded in a 384-well format, with silver nitrate acting as an ionization control. The in vitro sedimentation, diffusion, and dosimetry (ISDD) model was used to estimate the cellular delivered dose. After 24 hrs of treatment, cells were live-labeled with MitoTracker (mitochondria), fixed, permeabilized and labeled with Hoechst-33342 (nuclei), SYTO14 (nucleoli) and fluorescent conjugates of concanavalin A (ER), phalloidin (actin cytoskeleton), and wheat germ agglutinin (Golgi/plasma membrane). A multiplexed cell viability and apoptosis assay was run in parallel. Cells were imaged using an Opera Phenix High Content Screening System and profiled using Harmony High Content Analysis software. Approximately 1700 morphological features were measured per cell and summarized to the well level for analysis. The ISDD model predicted that the fraction of AgNP deposited on cells increased with particle size and differed based on coating (branched polyethyleneimine (BPEI) > citrate > Polyvinylpyrrolidone (PVP)). Phenotypic profiling showed that all AgNP types affected the morphology of each organelle in a concentration-dependent manner, with many effects observed below the threshold for cytotoxicity. The pattern of changes in cell morphology differed across capping agents and silver nitrate. For example, the effect of citrate on mitochondrial texture was opposite that of other AgNP coatings. 60 nm PVP had fewer effects on cell morphology than other coatings, yet showed apoptosis at an estimated delivered dose as low as 1.45 µg/ml. In summary, high content imaging assessed the cellular toxicity of AgNPs, and identified signature morphology patterns that differentiated between three common AgNP coatings. Further, this screening method may inform subsequent assay selection by highlighting the intracellular regions effected by AgNPs of interest. *This abstract does not necessarily reflect USEPA policy.*

**MP248 Cross-species compartmental modeling of selenium in fishes exposed to selenomethionine via ingestion**

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Selenium (Se) is a trace element and essential nutrient for almost all forms of life. However, it is also a common component and/or contaminant in industrial and agricultural activities and effluents, and can be toxic when encountered in excess. Where aquatic environments are inundated with Se the biota show increased Se concentrations, presumably in the bioaccumulative form of selenomethionine (SeMet), a naturally occurring organic methionine analogue. Many organisms that ingest SeMet show increased risk of toxicity commensurate with exposure, and oviparous animals show increased maternal transfer of Se to their eggs, which can cause significant teratogenicity of exposed embryos. Though the primary mechanism identified for SeMet toxicity is the redox cycling of its metabolite methylselenol, not all fish species exposed to SeMet manifest symptoms of oxidative stress, suggesting alternate toxicity pathways may exist. The non-specific substitution of SeMet for methionine presents a vast number of ways in which SeMet might disrupt animal physiology, including an enhanced propensity of protein oxidation, disruption of methylation cycles, and alternative regulation of SeMet and methionine molecules and metabolites, including homocysteine, which could have broad ramifications for numerous animal systems. Cross-species systemic partitioning models can therefore be employed to characterize the relative organ sensitivities within and across species, which can then be compared with species specific SeMet pathologies to help identify the broader suite of mechanistic pathways affected by SeMet. This information can then be used to direct targeted explant and/or in vitro studies, which will enhance our mechanistic understanding of SeMet toxicity, and lead to better Se risk assessment while reducing expenditure of time, money, and animal life.

**MP249 Future Water Quality Challenges to Aquaculture**

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Concerns about water quantity and quality are increasing due to climate change and population growth. Climate change is driving changes in evapotranspiration and precipitation patterns. This is exacerbated as population growth, particularly in arid and semi-arid regions, increases water extraction and consumption. Aquaculture is growing both globally and within the U.S. (worldwide 9.2% $yr^{1}$ 1990-2000 & 6.2% $yr^{1}$ 2000-2012; vs U.S. agriculture 1.86% $yr^{1}$ 1990-2000) (FAO, 2014; Ball et al., 2017). Freshwater aquaculture in the U.S. is largely dependent on surface water (80.78%) and ground water sources (19.32%) (Maupin et al., 2010). Surface water sources are increasingly dominated or dependent on treated wastewater effluent. Wastewater effluent generally contains trace levels of anthropogenic compounds, typically referred as contaminants of emerging concern (CECs), for which our knowledge of their impacts is still evolving. Therefore, the introduction of CECs in aquaculture from surface waters influenced by wastewater effluent is a potential concern for cultured fish health as well as for humans when consuming farmed fish. To improve our understanding of future water resource quality and quantity in relation to the feasibility and safety of aquaculture, wastewater effluent and surface water data was collected, consolidated and analyzed to examine trends in surface water quantity and quality. This data was collected from databases maintained by the United States Geological Survey (USGS) and United States Environmental Protection Agency (USEPA). Next, trends in HUC Wastewater effluent to stream flow dilution was statistically analyzed towards the water quality and fresh water availability in USA, which is projected for next five years using Prophet model in R. The results were then visualized using GIS to explore future water scenarios in the USA.

**MP250 T1000: identification of toxicogenomics gene sets for improved risk assessment of environmental toxicants**

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BACKGROUND: With thousands of untested exposure chemicals in the environment, selection of a responsive set of genes can facilitate high-throughput screening (HTS). However, while several gene lists were published based on a genome-wide expression profiling, a toxicogenomics signature is not well-addressed. OBJECTIVES: To find a set of globally responsive genes representing a reference signature for efficient HTS of potential effects of toxic chemicals. METHODS: Here we developed a novel machine learning approach to systematically characterize genes that are highly responsive in toxicogenomics studies. Toxicogenomics-1000 (T1000) gene signatures are derived based on in vitro and in vivo experiments from human and rat. RESULTS: A list of 1000 ranked genes (T1000) has been generated as a reference toxicogenomics signature. External evaluations have shown that T1000 achieved a correlation of about 90% of time match apical outcomes versus 24 experimental groups. When compared to baseline gene sets mapped using Limma and L1000 on the Rat Genome 230 2.0 Array, T1000 achieved a relative improvement on the $F_1$Score by 6% and 17%, respectively. The applicability of T1000 was demonstrated in a wide range of toxicological endpoints with 7,142 expression profiles. CONCLUSIONS: T1000 reference gene list represents a highly responsive signature for toxicogenomics studies. The achieved patterns of responses in genes and related pathways shows that T1000 can improve current HTS assays in toxicogenomics studies to facilitate prediction of adverse health outcomes.

## Terrestrial Toxicology, Ecology and Stress Response

### MP252 Assessing seasonal factors influencing parasitic infection in northern bobwhite quail of the Rolling Plains, TX using a mobile laboratory platform

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Northern bobwhite quail (*Colinus virginianus*), a popular gamebird, have been declining over recent decades in the Rolling Plains ecoregion of the United States. Previous investigations have revealed a high prevalence of eyeworms (*Oxyspirura petrowi*) and caecal worms (*Aulonocephalus pennula*) in quail from this area. This prevalence has been observed alongside numerous reports of bobwhites flying into objects as well as up to 100% infection in some areas of this ecoregion, prompting a need to better understand host-parasite interaction and other factors that influence infection. In this study, a mobile research laboratory is used to detect and quantify infection levels in bobwhite at three field sites in the Rolling Plains. Due to the life cycle in which these parasites operate, parasite eggs are expelled through the feces, consumed by an insect intermediate host, and subsequently eaten by a bobwhite. To detect the parasite eggs, cloacal swabs and feces from bobwhites are collected at each field site, then DNA is extracted and run using highly specific and accurate quantitative PCR (qPCR) to non-lethally assess infection and release unharmed the sampled quail. This data, coupled with data from previous years, provides valuable insight in understanding potential climactic factors that can impact parasite activity. Additionally, this research can identify the best timing of future anthelmintic treatment application in congruence with nematode diapause, a phenomenon that is suggested to hinder the effectiveness of treatment. These methods can also be integrated into current investigations of the role of parasites in bobwhite stress responses and possibly enhanced susceptibility to chemical exposure. Nevertheless, with the current use of a mobile research laboratory and an understanding of influential climactic factors, future effective treatment methods can be implemented to contribute to successful mitigation of parasitic infection and attempt to stabilize bobwhite populations in the Rolling Plains.

### MP253 Assessment of pesticide impacts on soil microbial viability using fluorescence, carbon dioxide, and nitrate assays

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Over 5 billion pounds of pesticides are used worldwide each year. Assessment of their impacts on soil microbes is essential as soil microbial functions such as $CO_2$ production and nitrogen cycling are critical to crop production and soil sustainability. Microbial viability in the presence of some common pesticides was assessed using fluorescence spectroscopy with nucleic acid stains, quantification of nitrate using ion chromatography, and titration of $CO_2$ evolved in respiration chambers. Glyphosate, imidacloprid, and 2,4-dichlorophenoxyacetic acid all showed a reduction in the ratio of live/dead soil bacteria when compared to untreated liquid soil cultures, with as much as 58% (imidacloprid) of the bacteria classified as nonviable. Monitoring of nitrate levels produced in nitrifying cultures showed significant reduction in nitrate production in the presence of either malathion or 2,4-D with an 8-fold reduction (from 242 to 28 mg/L) for malathion used at recommended dosages. Glyphosate spiked soil column studies showed anywhere from 28-55% (95% confidence) reduction in nitrate production but returned to normal nitrate production 3 weeks after application. $CO_2$ evolution from 5-day liquid soil cultures was reduced by as much as 99% (95% confidence) in the presence of malathion where 2,4-D treated cultures were much more inconsistent in response and showed no statistically significant effect. Continued assessment of the effects of pesticides on soil microbes is essential in order to develop prudent practices for their uses and to minimize impacts on soil microbial health.

### MP254 Assessment of Phytotoxicity of Ammonia on Radish (*Raphanus sativus*): Comparative Study on Phytotoxicity According to Chemical Accident Scenarios

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There are differences in the damage of the terrestrial ecosystem caused by chemical accidents depending on accident chemicals and type of chemical accidents. It is necessary to establish ecotoxicity database of accident chemicals through studies for ecotoxicity evaluation to accurately estimate the damage of the terrestrial ecosystem caused by chemical accidents. The terrestrial ecosystem can be exposed to ammonia, which is regulated as a chemical with a high frequency of accidents by Korea Ministry of Environment (MOE), through two pathways; i) inflow of aqueous ammonium hydroxide ($NH_4OH$) into the soil due to spillage and ii) deposition of gaseous ammonia ($NH_3$) due to explosion. Phytotoxicities of ammonia on radish (*Raphanus sativus*), representative field crop in Korea, according to chemical accident scenarios were assessed and compared. *R. sativus* was grown to 2 ~ 4 true leaf stage in non-contaminated condition according to the OECD guideline 227 and then exposed to aqueous $NH_4OH$ or gaseous $NH_3$. The number of survivors, biomass, chlorophyll content, and visible damages on the leaves were measured after four weeks of cultivation. The toxicity values (LOEC, NOEC, and $EC_{50}$) were calculated using SAS 9.3 software. Relatively sensitive endpoints were selected through comparison of phytotoxicities according to chemical accident scenarios. The results of this study will provide the appropriate ecotoxicity data of ammonia according to the exposure pathway when the terrestrial ecosystem is exposed to ammonia due to chemical accidents. Acknowledgement: This subject is supported by Korea Ministry of Environment(MOE) as "The Chemical Accident Prevention Technology Development Project."(2016001970003)

### MP255 Chronic toxicity of four insecticides to *Apis melifera* larvae reared in vitro

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Honey bees can be exposed to pesticides and environmental toxic compounds while foraging in diverse environments. In addition to honey bees, larvae can be exposed to various pesticides from residues contained in pollen, nectar, and wax in the hive. However, at present, side effects and toxicity of pesticides on larvae are poorly investigated. The objective of this study is to assess the chronic toxicity of four insecticides to honey bee larvae using an in vitro rearing method adopted by OECD GD NO.239. Carbaryl, chlorpyrifos, cypermethrin, and diazinon were chosen for this study because they are the most widely used insecticides for agricultural area in Korea and also known to be detected in nectar, pollen, and wax. The $LD_{50}$ values of carbaryl, chlorpyrifos, cypermethrin, and diazinon at D8 were 1.00, 0.07, 0.17, and 0.43 ug/larva, respectively; at D15 were 1.13, 0.06 0.17, and 0.40 ug/larva, respectively; and at D22 were 0.88, 0.05, 0.13, and 0.35 ug/larva, respectively. The toxicity of the four insecticides to honey bee larvae from most to least toxic was chlorpyrifos > cypermethrin > diazinon > carbaryl. Various mortality symptoms at sublethal concentration like melanizing, failed molt, failed adult molt, and deformed adults were also observed and recorded.

**MP256 Comparing the effects of nanoparticles ZnO, CuO, and TiO2 nanoparticles on reproductive function and gene expression in *Caenorhabditis elegans***

*T. Thornburg, X. Pan, East Carolina University / Biology*

The use of metal oxide nanoparticle in forms of Zinc, Titanium and Copper oxides has been increased nowadays in various consumer products. However, the toxicity information regarding exposure to these nanoparticles are still limited. This study focuses on investigating their impacts on reproductive functions and comparing of toxicity between different nanoparticles with different metal components. The model organism *Caenorhabditis elegans* (*C. elegans*) was used and the following experiments are performed: 1) Characterize effects of exposure to a range of environmentally relevant concentrations of ZnO, CuO, $TiO_2$ on reproductive function. Reproductive defects include alterations in brood size, abnormalities in spermatogenesis, oogenesis, embryogenesis, and abnormality in offspring development and behaviors. And 2) Evaluate effects of exposure to different metal oxide nanoparticles on gene expressions by quantitative real-time PCR. Our preliminary observations using florescence microscope following DAPI staining have identified the changes in gonadal morphology, chromosome integrity, and mitosis and meiosis progression following exposures. The gene expression analysis are ongoing. Changes in worm locomotion behaviors following exposure have also been characterized. This study using the *C. elegans* model will guide validation studies in vertebrate animals.

**MP257 Effects of PCB exposure on thyroid hormone levels in serum of dogs and cats**

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Thyroid hormones (THs) such as 3,3’,5-triiodo-L-thyronine ($T_3$) and L-thyroxine ($T_4$) are essential for the regulation of growth and development. Recent studies reported that polychlorinated biphenyls (PCBs) and their hydroxylated metabolites (OH-PCBs) affect the TH homeostasis. In our previous studies, concentrations of PCBs and OH-PCBs in pet cat serum showed significant negative correlation with THs. However, there are no in vivo evidences on the adverse effects of PCB exposure on thyroid hormone homeostasis of pet animals. Thus, the present study aimed at investigating the effects of PCB exposure on TH homeostasis in dogs and cats by measuring serum TH levels following a single dose of 12 PCB congeners (0.5 mg/kg of each congeners: CB18, 28, 70, 77, 99, 101, 118, 138, 153, 180, 187 and 202) and by examining the relationships with levels of PCBs and OH-PCBs. Concentrations of PCBs and OH-PCBs in dog serum were higher than those in cat serum after 24 hours exposure to the same dose of PCBs. In dog, free T4 level increased with PCB exposure and was positively correlated with the levels of T4-like OH-PCBs including 4-OH-CB107 and 4-OH-CB202. This was probably caused by competitive binding between T4 and T4-like OH-PCBs to TH transport proteins. Additionally, total T4 and total T3 levels in PCB-exposed dogs lower than in the control group and were negatively correlated with PCB concentrations. It was reported that PCB exposure induced the decrease of total T4 in serum due to induction of UDP-glucuronosyl transferase (UGT) and accumulation of T4 in liver. This result implies that PCB exposure enhanced TH excretion in dog by increasing TH uptake and UGT activity in the liver. In contrast, TH levels in cat sera did not change with PCBs exposure. This result can be explained by the lower levels of PCBs and T4-like OH-PCBs compared with dogs and very low activity of UGTs in cats.

**MP258 Gallic acid protects against bisphenol A-induced alterations in the cardio-renal system of Wistar rats through the antioxidant defense mechanism**

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Bisphenol A (BPA) is a small molecular weight endocrine disrupting chemical (EDC) that is used in the production of plastics with deleterious effects on various body systems while gallic acid (GA) is a known antioxidant capable of ameliorating EDC-induced perturbations. In this study, adult male rats (180 ± 5g) were divided into four groups of eight rats each: Group A (Control rats): 0.2 ml of corn oil; Group B (GA-treated rats): 20 mg/kg/day GA (dissolved in distilled water); Group C (BPA-treated rats): 10 mg/kg/day BPA suspended in 0.2 ml corn oil; Group D (BPA+GA-treated rats): BPA (10 mg/kg/day) with a concomitant GA (20 mg/kg/day). All treatments were orally administered for 14 days. BPA induced significant decrease in systolic, diastolic and mean arterial blood pressure while causing a significant increase in heart rate in the rats. It significantly raised both renal and cardiac reactive oxygen species and depleted the antioxidant system. There were also significant increases in serum myeloperoxidase, nitric oxide, urea and creatinine in the BPA-treated rats. Lesions of the heart and kidney including inflammation, vascular congestion and erosion of epithelial cells were also observed in the BPA-treated rats. However, the concomitant treated with GA ameliorated all the BPA-induced alterations of the cardio-renal system. Hence, low dose of GA serves a protective function against BPA-induced toxicity of the heart and kidney.

**MP259 Impact of Seasonal Temperature Variation on Pesticide Fate and Metabolism in Urban Landscapes**

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The United States contains 50 million acres of managed turfgrass. In order to maintain this turfgrass acreage, approximately 30 million kilograms of pesticides are applied to urban landscapes, representing more than 20% of the total pesticide usage in the U.S. The herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D) is among the most commonly used pesticides for turfgrass management in urban landscapes. Although the fate and breakdown of 2,4-D has been well documented, assessments of its transformation products (TPs) under varying environments have not yet been clearly elucidated. Biotic mechanisms are considered to be the primary force of pesticide degradation within soil ecosystems and can be largely influenced by a broad range of environmental factors. Specifically, temperature variations are a central environmental factor that directly influences the soil microbiome structure and activity which may indirectly alter pesticide degradation networks and in the formation of multiple TPs with varying toxicological effects. Within this context, there is limited knowledge regarding pesticide-microbe interactions in urban soils and their metabolic pathways under the varying environmental conditions that 2,4-D is typically applied. In this study, we investigated the formation of 2,4-D metabolites under seasonal temperature fluctuations and evaluated how these variations influence microbial-pesticide interactions. Growth chamber and field studies were performed on turfgrass samples to assess 2,4-D’s fate and movement under both natural and controlled environment conditions. Pesticide extractions were performed using a modified multiresidue Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) solid-phase extraction method. Electrospray ionization Liquid Chromatography/Mass Spectrometry analytical protocols were also developed for the detection of 2,4-D and its respective TPs in turfgrass soil samples. TP formation in response to seasonal turfgrass-associated microbial community changes was also assessed through microbiome analysis and targeting the 2,4-D degrading gene, TfdA, commonly found in soil bacteria.

### MP260 In-Vitro Evaluation of Potential Hepatotoxicity Induced By Detergent-Processed Cassava Product

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Toxic effects of cassava-based products on humans have been established. The recent use of detergent in processing cassava into food products such as *fufu* (a local delicacy Nigeria), may exacerbate its toxic effect. This study determined the hepatotoxicological effect of detergent-processed *fufu* on Wistar rat. The liver is a heterogeneous organ which is involved in detoxification of xenobiotics and is highly susceptible to injury from these substances. With the increase in chronic diseases among the Nigeria growing population, there is need to look beyond pathogenic and infectious diseases and focus on public health nutrition. Rat models were used in assessing the degree of damage caused on the haematological and hepatological parameters. Graded concentrations (7.5mg/L, 15mg/L and 22.5mg/L) of detergent was used in fermenting cassava for making the *fufu* which was used in the study. Wistar rats were given 7 feeding treatments (TRT): TRT I – positive control (commercial rat feeds only); TRT II – positive control (*fufu* only); TRT III – negative control (detergent only), TRT IV – designated control (*Fufu* bought from the open market), TRT V – VII (*fufu* plus graded concentrations of detergent). Liver marker enzymes were assayed in the plasma and liver homogenate, blood parameters were also analysed after four weeks of treatment.. Levels of marker enzymes such as Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Alkaline phosphatase (ALP) were increased significantly across all the detergent treatment groups and TRT II and IV, while plasma ALP only increased in TRT III . No significant difference was observed in the liver homogenate except in AST which showed increase across all treatment groups except group VIII. There was no significant difference in the blood parameters across all groups. The present observations suggested that consumption of detergent-processed *fufu* poses risk to the liver while the blood parameters remain unaffected

### MP261 Individual and Combined Treatment Effects of Pesticides, Fertilizers, and Salt on Larval Amphibian (*Lithobates sphenocephala*) Growth and Stress Levels

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Human activities have introduced a variety of chemicals, including pesticides, fertilizers, and salt, into the environment which have had deleterious effects on the organisms inhabiting these areas. Amphibians are especially susceptible to absorption of chemical pollutants. To determine the possible combined effects of these chemicals on amphibian development and stress levels, Southern leopard frog (*Lithobates sphenocephala*) larvae were exposed to one of eight individual or combined treatments of atrazine, ammonium nitrate fertilizer, and sodium chloride salt. Stress levels, indicated by release of the stress hormone corticosterone, were measured pre-metamorphosis at week 8 of development and again at 24 hours post-metamorphosis. Water hormone samples were processed using ELISA kits from Cayman Chemical to analyze corticosterone levels. Changes in tadpole growth were determined by surface area measurements taken from biweekly photographs analyzed with IMAGEJ software. The combined chemical treatment of atrazine, salt, and fertilizer had a significant interactive effect of increasing stress levels prior to metamorphosis ($p=0.003$), but this effect was no longer apparent 24-hours post-metamorphosis ($p>0.05$). By the end of larval development, tadpoles exposed to nitrogen had significantly larger surface area ($p=0.035$). Tadpoles exposed to atrazine had significantly lower changes in growth throughout larval development ($p\leq 0.001$), however, the frogs in the atrazine treatment metamorphosed faster ($p=0.002$). As amphibians are exposed to multiple chemicals simultaneously in the environment, assessing the effects of a combination of contaminants is necessary to improve application strategies and ecosystem health.

### MP262 Investigating effects of sodium benzoate on the *Caenorhabditis elegans* model

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Clinical observation has proposed the linkage between the occurrence of obesity and slow weight loss with daily exposure to common food additives such as benzoic acid. However, well-controlled laboratory experiments on this topic have been lacking; the dose-response relationship of benzoic acid and obesity occurrence and/or slow weight loss has not been established; the potential mechanism of such linkage has not been explored. This is an important point because the EPA does not have a current limit on Sodium Benzoate, and the FDA limit has not been updated in over two decades. The insulin-signaling and fatty acid synthesis pathways in the model organism *Caenorhabditis elegans* (*C. elegans*) are highly conserved with higher organism including humans and thereby has been widely utilized to study obesity and aging related mechanisms. Scientific literature links the insulin pathway of *C. elegans* to growth, development, longevity, behavior, and metabolism in the organism (Murphy 2013). *C. elegans* is also a perfect model for exploring the genetics of fat storage (McKay 2003). Thereby, I plan to use *C. elegans* as a model organism to study the effects of benzoic acid exposure on fat storage and on the gene expression of major players in insulin signaling pathway. I hypothesize that long-term exposure to benzoic acid will lead to altered fat storage, and gene expressions in the insulin-signaling and fatty acid synthesis pathways. To test this hypothesis *C. elegans* will be exposed to different concentrations of benzoic acid, and effects will be observed over a long period of their life span. The fat storage of the organism will be monitored using histological staining and microscopic observation and then quantified using imaging software. Genes in the insulin-signaling pathway will be selected and their expression profile will be tested. Once benzoic-acid response genes are identified, mutant strains will be used to test their sensitivity to benzoic acid exposure and further reveal the gene functions in response to benzoic acid exposure. Preliminary data suggest that sodium benzoate has a dose dependent effect on the growth of *C. elegans*. The cause of this growth issue will be explored in further research.

### MP263 Parasite Burdens in Northern Bobwhite Quail from the Rolling Plains Ecoregion of Texas and Potential Interactions with Agricultural Pesticides

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A concern that has been increasing in recent years is the impact of parasites in wildlife and how they interact with natural and anthropogenic stressors. Recently, it has been suspected that *Aulonocephalus pennula* and *Oxyspirura petrowi* are playing a role in the decline of the Northern bobwhite quail (*Colinus virginianus*), a popular and economically important gamebird in the Rolling Plains ecoregion of Texas. From January 2017 to March 2018, a total of 154 bobwhite were assessed for the presence of parasites. During this time, we documented some of the highest parasite loads of *A. pennula* and *O. petrowi* recorded in quail in over six years of continuous monitoring with concurrent population drops. This finding was concerning as increased parasite burdens have the potential to suppress the immune system and may explain the increased prevalence of even a third parasite, *Physaloptera* sp., in our samples. The cause for these increased infections remains to be determined and the mechanism for this increase requires further investigation. However, high parasite burdens have also been associated with increased levels of contaminants within a host. The chance of bobwhites being exposed to a contaminant is likely in the Rolling Plains as this region is one of the major producers of cotton in Texas, and cotton utilizes agricultural pesticides, such as neonicotinoids, and may expose bobwhites. The potential interactions of contaminants in the Northern bobwhite quail, such as agricultural pesticides, and parasites in bobwhite will be discussed, particularly in light of the increased prevalence of parasites in this region.

**MP264 Pilot study on the toxicological assessment of organohalogen compounds in pet cat (*Felis catus*) serum using metabolomics approach**

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Domestic pets such as dogs and cats share living environments with humans. Therefore, they are exposed to various contaminants, including PCBs and PBDEs in their immediate surroundings, which raise concerns about health risks. Recent studies have reported elevated PBDE levels in the sera of cats. Metabolome analyses performed in toxicological studies provide information about the biological systems and toxicological pathways. The present study determined the levels and accumulation patterns of PCBs, PBDEs, and their metabolites (OH-PCBs and OH-PBDEs) in the blood of pet cats (male= 8, female = 8)collected from a veterinary hospital in Japan. Furthermore, in order to assess the effects of contaminants in pet cats, we also examined relationships between the levels of organohalogen compounds and serum Metabolome levels analyzed using LC-MS/MS. Median concentrations of PBDE (wet weight) in the cat serum (630 pg $g^{-1}$) were higher than that of PCBs (290 pg g−1). Especially, deca-BDE was the predominant homologue, accounting for 60% of total PBDEs concentrations. In the PCB congeners, CB153, 138, 180 and 118 were the predominant homologues. We analyzed polar metabolomics profiling using LC-MS/MS that covers major metabolic pathways. The OPLS analyses found 13 metabolomes (8 increases and 5 decreases) were significantly associated with PCBs concentrations, and 17 metabolomes (15 increases and 2 decreases s) were significantly associated with PBDEs concentrations. Enrichment analysis suggested that PCBs concentrations in the cat serum were associated with the glutathione metabolism, glycerolipid metabolism, pentose phosphate pathway, phenylalanine, tyrosine and tryptophan biosynthesis. In addition, PBDEs concentrations were suggested to be associated with the alanine, aspartate and glutamate metabolism, and pyrimidine metabolism. The activations of pentose phosphate pathway and glycolysis associated with the exposure of these PCBs and PBDEs may be related to the activation of drug metabolizing enzymes (cytochrome P450). Inhibition of GSH and GSSG was observed for PCBs exposure level. Inhibition of GSH and GSSG are known to be sensitive markers for oxidative stress. Therefore, these results was suggested that oxidative stress production accompanying the induction of CYPs.

**MP265 Small-scale spatial variation of methylmercury concentrations in sediments and invertebrates from intertidal wetlands**

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Periodic wetting and drying in tidal wetlands re-oxidizes the sulfur and iron in sediments that act as electron acceptors for sulfate- and iron-reducing bacteria known to facilitate the conversion of inorganic mercury (Hg) to methylmercury (MeHg). Wetting and drying frequency and associated sediment re-oxidation is a function of wetland elevation and hydrology. Therefore, small-scale spatial differences in MeHg production can occur in less saturated wetland habitats like channel edges compared to marsh interior habitats that experience more frequent wetting and drying. We compared seven measures of sediment biogeochemistry, including sediment MeHg concentrations, between channel edge and interior habitats in three tidal wetlands along the Petaluma River in San Francisco Bay, CA. We also assessed MeHg concentrations of invertebrates (Amphipoda, Gastropoda, and Arachnida) collected near sediment sampling locations. Sediment MeHg concentrations were significantly greater in interior habitats than along channel edges ($p < 0.001$), and the concentration of inorganic reactive mercury (RHg) contributed significantly to a linear discriminant function, indicating that the RHg pool was more depleted in interior habitats than at the channel edge ($p < 0.001$). We did not detect significant effects of either sediment MeHg (p = 0.95) or percent MeHg (p = 0.96) on invertebrate MeHg concentrations. We also did not find significant differences in invertebrate MeHg concentrations between habitats ($p = 0.39$), suggesting that invertebrate MeHg was not directly associated with surface sediment MeHg concentrations. These findings may have resulted from spatial or temporal mismatches in sediment and invertebrate sampling approaches. Scrapers and detritivores like Gastropoda and Amphipoda forage in the top few millimeters of sediment, where they may be exposed to different MeHg concentrations than those measured in our 2 cm sediment samples. Last, invertebrate uptake of MeHg from sediments is not instantaneous and assimilation rates are unknown for the invertebrates in our study. Thus, temporal lags could have prevented us from detecting a relationship between MeHg concentrations in sediment and invertebrates. Since hydrological patterns can drive wetland MeHg production at small spatial scales, future studies aimed at understanding spatial variation in sediment MeHg and its uptake into food webs should consider the spatial and temporal concordance of their sampling design.

**MP266 Sublethal Effects of Chronic Exposure to CdO or PbO Nanoparticles or Their Binary Mixture on the Honey Bee (*Apis millefera* L.)**

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Cadmium and lead-based nanotechnologies are increasingly used in agricultural, industrial and biological processes; however, potential adverse effects of nanomaterials on honey bees had not been assessed. In this study, effects of exposures to sublethal concentrations of PbO and CdO nanoparticles (NPs), either separately or in combination on honey bee (*A. mellifera*) workers were assessed. Honey bee workers were orally exposed for nine days under laboratory conditions to sublethal concentrations (10% of $LC_{50}$) of CdO (0.01 mg $ml^{-1}$) and PbO (0.65 mg $ml^{-1}$) NPs either separately or combined. Effects on survival, feeding rate, activity of acetylcholinesterase (AChE), and expression of stress-related detoxifying enzymes were quantified. Survival and feeding rates decreased particularly in bees fed sugar syrup containing CdO NPs and binary mixtures of NPs of both metal oxides. Expressions of genes involved in detoxification of xenobiotics were affected by various combinations. Expression of catalase was 13.6-fold greater than control in bees consumed sugar syrup diet containing binary mixture of sub-lethal concentrations of both CdO and PbO NPs. AChE activity in honey bee heads inhibited by 3.8-, 3.0- and 2.8-fold relative to control, respectively in response to exposure to Cd or / and Pb oxides NPs compared to controls, which indicates potential neurotoxic effects of these NPs on honey bees. CdO NPs exhibited the greater potency on honey bees. Overall, honeybee workers suffered detrimental effects when they were exposed to sublethal concentrations of CdO or/and PbO NPs.

## Birds Under Stress: Impacts of Chemical Exposure and Environmental Changes

### TP001 Mercury concentrations vary within and among body feathers in birds: A critical evaluation and guidelines for feather use in mercury monitoring

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Feathers are an easily and non-lethally collected tissue that is extensively used to represent mercury (Hg) contamination in birds. Yet, few recommendations exist that provide guidance on how to appropriately design, sample, process, and interpret Hg in feathers. This is important because Hg concentrations can vary substantially within and among individual feathers from the same bird. Thus, it is difficult to interpret feather Hg concentrations and laboratory methods may bias results. We conducted a series of 5 experiments to test Hg variability within and among body feathers (breast, back, head) and used the results to develop a tool to guide future feather sampling designs and processing for Hg analysis. We found that 1) Hg concentrations varied extensively among whole feathers within individual birds from 5 orders (1.4-8 fold different), with a coefficient of variation (CV) 9-68%; 2) to obtain a measured Hg concentration within 10% of a bird's true average feather Hg concentration, 4 or 21 feathers were required when the feather CV was 10% or 20%, respectively; 3) Hg concentrations in the feather vane were 44% higher than the rachis and 111% higher than the calamus, indicating that methodological differences in processing can influence the resulting Hg concentrations; 4) using scissors or scissors + liquid nitrogen to homogenize feathers decreased the variability in Hg concentrations among aliquots relative to whole feathers, but using liquid nitrogen introduced a bias by underestimating a bird's true average feather Hg concentration; and 5) the number of whole breast feathers that could be run per sample boat was related to mean species body mass ($R^2$=0.80; n=70 species) due to the capacity of a sample boat for Hg determination. Our tool can be used to guide the strategic design of studies using feathers to quantify Hg contamination in birds and includes 3 critical components: A) variability of feather Hg concentrations within an individual, B) desired accuracy of the measured Hg concentration, and C) feather size. Our results suggest a general rule that, if the goal is to be within 10% of a bird's true average feather Hg concentration by running only 1 sample boat, Hg variability among feathers must be low (CV=10%) and the bird can be larger (≤251g) because only 4 whole feathers need to fit in 1 boat, whereas when Hg variability among feathers is higher (CV=20%) the bird must be smaller (≤16g) because more whole feathers (≥21) need to fit into 1 boat.

### TP002 Comparison of metal exposure and egg characteristics in great tits inhabiting four metal-polluted environments in Europe

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Metal-polluted environments represent an important challenge for passerines and may affect the maternal investment into egg quality. Toxic metals may impair the eggshell structure as they can alter Ca absorption and the homeostasis and function of Ca. Great tits (*Parus major*) lay white eggs speckled with brown protoporphyrin pigment spots. Toxic metals may also affect this eggshell spotting pattern by depressing the heme synthesis and inducing protoporphyrin accumulation in blood, which may increase its deposition on eggshells in polluted areas. Our aim is to assess the effect of metal exposure on early-stage reproduction in free-living great tits inhabiting different metal-polluted environments in Europe. For this purpose, we carried out an extensive sampling in 2016 to collect eggs and nestling feces for assessing metal exposure in great tits breeding in industrial/urban sites and control environments in Belgium, Finland, Hungary and Portugal. We evaluate different egg characteristics that can be sensitive to metal effects such as eggshell thickness and pigmentation pattern. Generally, nestlings in the industrial/urban environments showed higher metal concentrations in feces, except for Portugal. Nestlings in Belgium were exposed to the highest metal levels. The eggshell index was slightly lower in the polluted site compared to the control site in Belgium while the opposite trend was found in Hungary, maybe due to the better Ca availability in the Hungarian polluted site. Although birds in industrial/urban environments were exposed to higher metal levels, the current pollution load is not affecting the eggshell quality. Eggs in the polluted environments showed higher spot intensity in Belgium, Hungary and Portugal, whereas neither effects on spot distribution nor size were found. Ca levels and toxic metals showed an interactive effect on spot intensity, suggesting that eggshell protoporphyrin-spotting intensity may be affected depending on the balance between metal pollution and Ca availability. Acknowledgements: We thank J. Nurmi, M. Rainio, L. Millán, T. Koivisto, P. Scheys, G. Eens and R. Pinxten. This study was financed by the Academy of Finland (project 265859 to T.E.), UTUGS (P.S.-V.), FCT (SFRH/BPD/99394/2013 to R.A.C.), FWO-Flanders and University of Antwerp (M.E.), Hungarian Scientific Research Fund (OTKA, PD100304) and Bolyai János Research Fellowship (MTA) to R.H., and *Fundación Séneca* (20031/SF/16 to S.E.).

### TP003 Reviewing and Using Current Toxicity Literature to Derive an Aquatic-Dependent Avian Tissue Residue Value (TRV) for Mercury

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Mercury contamination of aquatic ecosystems is widespread and bioaccumulation and biomagnification of methylmercury occurs through the aquatic food web. Aquatic-dependent bird species appear to be particularly sensitive to mercury exposure, experiencing effects such as reproductive impairments at environmentally relevant concentrations (Burgess and Meyer 2008; Brasso and Cristol 2008). Both the Great Lakes Initiative (GLI 1993) and Mercury Study Report to Congress (MSRC 1997) included derivations of an avian Tissue Residue Value (TRV). During the development of both the GLI and the MSRC, the results of several mallard studies (Heinz 1974, 1976a, 1976b, and 1979) were determined to be the most appropriate toxicity endpoints for the derivation of an avian TRV. These mallard studies demonstrated that exposure to dietary methylmercury reduced reproductive success at 0.5 ppm methylmercury (Heinz 1979). Both the GLI and MSRC utilized this toxicity endpoint as a lowest adverse effect level (LOAEL), which was then adjusted by an uncertainty factor of 6 (GLI 1993) or 3 (MSRC 1997) to derive TRVs of 13 µg/kg and 26 µg/kg, respectively. In the 20 years since the development of the GLI and MSRC, mercury lab and field-based toxicity studies focused on a wide diversity of bird species have provided additional information into the potential effects of mercury and the relative sensitivity of birds. The current work includes review of the latest scientific literature on the effects of mercury on aquatic-dependent birds and application of new data to develop updated toxicity values for some aquatic-dependent bird species.

### TP004 Role of oxidative status on growth and fledging success in great tit nestlings: A calcium supplementation experiment in a metal-polluted area

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The increased metabolic activity associated with rapid growth may cause oxidative stress that may affect physiological and life-history traits such as nestling size, growth or fledging success. Thus, nestling development is a demanding period that may be even more challenging when birds inhabit environments suffering from metal pollution and reduced food quality and quantity. Our aim is to explore the effects of antioxidant molecules and oxidative damage biomarkers on nestling size, growth and fledging success taking into account the metal exposure in great tit (*Parus major*) nestlings. During a Ca-manipulation experiment, great tits were supplemented with Ca or not supplemented (Control group) in a metal-polluted and a control area in SW Finland. We measured oxidative stress biomarkers (GSH, GSH:GSSG ratio, GPx, GST, CAT, SOD, lipid peroxidation -TBARS- and protein carbonylation) in red blood cells. Our results showed that smaller nestlings showed higher TBARS levels, suggesting that nestlings suffering poorer condition have increased lipid peroxidation. However, the lack of differences in TBARS levels between zones shows that metal exposure seems to be low to directly produce oxidative damage to lipids. In addition, a higher growth rate was associated with higher TBARS levels in erythrocytes, showing that nestlings growing faster may have a weaker capacity to face oxidative damage. A higher growth rate was also associated with enhanced CAT activity and tGSH levels (in the polluted area), probably reflecting an up-regulation of those antioxidant molecules in response to the increased oxidative challenge in growing nestlings, particularly in those exposed to low doses of metals. Similarly, the fledging success increased with increasing CAT activity in the polluted area, which may reflect the necessity of the organism to enhance this enzyme to improve its probability to fledge in response to the increased oxidative stress in a situation of metal exposure and lower quality and quantity of food. Finally, increased number of fledglings was associated with decreased SOD activity, which could reflect higher oxidative stress in nestlings from larger broods due to sibling competition. Our study shows that antioxidant molecules and oxidative damage were related to nestling development, and the oxidative status, conditioned by metal pollution, may have consequences on growth and fledging success. *Acknowledgements*: Academy of Finland, UTUGS and *Fundación Séneca*.

**TP005 Occurrence of legacy pesticides, current use pesticides, and flame retardants in conservation areas**

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The prevalence and environmental adverse effects of persistent organic pollutants (POPs), likepolybrominated diphenyl ethers (PBDEs) and legacy pesticides, are a well-known problem. Since environmental threats such as global environmental change and pollution occur across borders and boundaries, protected areas are vulnerable despite their conservation status. To improve current conservation practices, a better understanding of how these threats move into protected areas is needed. Therefore, we examined the occurrence of legacy pesticides, current use pesticides (CUPs), and flame retardants in forests across Costa Rica and Uganda. We aimed to identify how regional differences and protected status influenced potential exposure to organic pollutants in protected areas. Air samples were collected using passive samplers with polyurethane foam (PUF) disks. Passive air samplers were deployed for ~3 months in Kibale, Uganda, and two biological field stations in Costa Rica. In Costa Rica, we selected La Selva Biological Station, which is surrounded by agricultural land used for bananas and pineapple and Las Cruces Biological Station, which is surrounded primarily by small-scale farming including coffee and cattle. Samples were analyzed for legacy pesticides, CUPs, and flame retardants, including PBDEs and organophosphate esters (OPEs) using gas chromatography mass spectrometry. Both in Costa Rica and Uganda, congeners BDE47 and BDE99 were the most abundant among PBDEs, TNBP, TCEP and TCIPP among OPEs, and chlorpyrifos among CUPs. The most abundant legacy pesticides were endosulfan and chlordane in Costa Rica and aldrin in Uganda. The median of legacy pesticides, CUPs, OPEs, and PBDEs at La Selva were 14.3 pg/$m^3$, 41.9 pg/$m^3$, 491 pg/$m^3$, and 3.75 pg/$m^3$, respectively, and 5.38 pg/$m^3$, 843 pg/$m^3$, 764 pg/$m^3$, and 7.35 pg/$m^3$ respectively, at Las Cruces. In Uganda, the median of legacy pesticides, CUPs, OPEs, and PBDEs were 46.5 pg/$m^3$, 6.91 pg/$m^3$, 247 pg/$m^3$, and 1.27 pg/$m^3$, respectively. Las Cruces and Kibale had the lowest levels of CUPs, but this was not the case for legacy pesticides, suggesting that long-term exposure to emerging and legacy pollutants occurs also in protected areas, regardless of protected status.

**TP006 Gulls as Bioindicators of Flame Retardant Emissions from UK Landfill**

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Waste streams are important reservoirs of legacy brominated flame retardants (BFRs). Several bird species use landfill as a foraging substrate and can be effective bioindicators of such persistent organic pollutants (POPs). We investigated BFRs and novel BFRs (NBFRs) across a UK landfill species-assemblage of gull taxa (*Larus* spp.) and report concentrations and profiles of polybrominated diphenyl ethers (PBDEs), hexabromocyclododecanes (HBCDD) and NBFRs in eggs of five gull species: black-headed gull *Chroicocephalus ridibundus*, common gull *Larus canus*, great black-backed gull *L. marinus*, European herring gull *L. argentatus* and lesser black-backed gull *L. fuscus*. Eggs were collected in Scotland (UK) from gull colonies located within 3 km of a landfill and those from reference sites. Aliquots (1 gram) of egg samples were spiked with internal standards prior to pressurised liquid extraction combined with an in-cell multi-silica clean-up. Clean extracts were concentrated to near-dryness and reconstituted in toluene containing recovery determination standards. PBDEs and NBFRs were determined via GC-EI/MS. HBCDD was determined by LC-MS-MS. Comparatively high arithmetic mean BFR concentrations in the eggs of great black-backed gulls ($\sum_7$PBDEs: 37.2 ng/g ww; HBCDD: 8.04 ng/g ww) and European herring gulls ($\sum$PBDEs: 34.3 ng/g ww; HBCDD: 4.5 ng/g ww) may be connected to their relatively wide foraging niches and abundance at the landfill study site, respectively. Black-headed gulls ($\sum$PBDEs: 5.71 ng/g ww; HBCDD: 1.24 ng/g ww) and common gulls ($\sum$PBDEs: 14.3 ng/g ww; HBCDD: 2.8 ng/g ww) were not observed foraging on this particular landfill, possibly explaining their relatively lower mean BFR egg burdens. Lesser black-backed gulls, which were observed using the landfill in small numbers, exhibited mean $\sum$PBDEs of 16.5 ng/g ww and mean HBCDD of 1.44 ng/g ww. Among NBFRs, we have detected only DBDPE at concentrations >1 ng/g ww, in five individual eggs of four species, i.e., black-headed gull (42 ng/g ww), common gull (27 ng/g ww), great-black-backed gull (484 ng/g ww) and European herring gull (two eggs: 3.3 and 4.8 ng/g ww). The relatively high concentrations in black-headed and common gulls (species that were not observed on the study landfill) may possibly suggest that landfill is not yet a major source of DBDPE. We believe that the DBDPE concentration reported in the great black-backed gull egg (484 ng/g ww) is the highest reported in wildlife to date.

**TP007 Avian red blood cells exposed to crude oil in vitro have damaged membranes and denatured hemoglobin**

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Population damage assessments of bird populations exposed to oil spills typically include counting the number of bird carcasses and visibly oil-coated birds, but often do not consider sub-lethal effects of ingesting crude oil. Other studies have shown that Heinz bodies (i.e., inclusions of denatured hemoglobin on red blood cell membranes) can be used as an indicator of oxidative damage to erythrocytes in birds exposed to crude oil. However, there is variation among bird species in whether crude oil exposure results in the formation of Heinz bodies and it is unclear whether the absence of Heinz Bodies indicates a lack of damage to erythrocytes.

In this study, we examined hemoglobin denaturation and membrane integrity in a species of bird that does not appear to form Heinz Bodies to discover if crude oil exposure causes oxidative damage to occur, even without the presence of Heinz Bodies. Erythrocytes were collected from zebra finches (*Taeniopygia guttata*) and exposed in vitro to six different concentrations of crude oil prepared as a PBS accommodated fraction (10, 20, 40, 100, 200, 400 ug/mL PAH) for 24 hours. To assess hemoglobin denaturation, we measured methemoglobin and t-parameter (i.e., hemoglobin degradation to hemichromes). We examined membrane integrity by measuring erythrocyte osmotic fragility. We found that exposure to crude oil causes hemoglobin degradation and impairs membrane integrity. Our study suggests that while Heinz Bodies are good indicators for oxidative damage for some bird species, erythrocyte damage can occur even in birds that do not form Heinz Bodies.

**TP008 Estimating Polychlorinated Biphenyl Risks to Birds: Dietary Doses, Egg Concentrations, and Body Weight Scaling**

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Although dietary dose-based toxicity reference values (TRVs) are typically used in food web models, it is well accepted that for many contaminants, egg concentrations can support more accurate risk estimates for birds. As such, tissue data provide a means to evaluate whether body weight scaling of TRVs for interspecies extrapolation improves the accuracy of risk estimates. We compared risk estimates for the polychlorinated biphenyl (PCB) mixture Aroclor 1254 in two avian species, gray catbird and great blue heron, using three measures of exposure and effect: 1) dietary doses (in milligrams per kilogram body weight per day) compared to dose-based TRVs, 2) dietary doses compared to body weight-normalized dose-based TRVs, and 3) modeled egg concentrations compared to egg-based TRVs. We used the same dietary assumptions and exposure point concentrations in each scenario. Due to variations of the aryl hydrocarbon receptor among species, gray catbirds exhibit high sensitivity to PCBs, and great blue herons are less sensitive. Dose and egg TRVs were developed from the scientific literature for each sensitivity group. Gray catbird TRVs were based on dose-response analyses of chicken toxicity studies, and great blue heron TRVs were based on no-effect exposures in mallards. An alternative egg-based TRV from a black-crowned night-heron study was also evaluated. Dose-based TRVs were normalized by body weight from the TRV species (chicken and mallard) to the wildlife species (catbird and heron) using the $BW^{3/4}$ scaling method employed by USEPA for human health risk assessment purposes. To model risks based on egg TRVs, PCBs in eggs were estimated from PCBs in diet. Paired diet and egg data were identified from the literature, and diet-to-egg bioaccumulation regressions were developed for Passeriformes (applied to catbird) and Pelecaniformes (applied to heron). The resulting risk estimates show better agreement between egg-based risks and dose-based risks determined using body weight scaling, with greater divergence when body weight scaling was not used. This analysis is part of a broader effort to encourage scientific consensus to move toward reinstating body weight scaling as an acceptable tool for wildlife TRV extrapolation. Meanwhile, the egg modeling approach provides a less controversial tool to achieve greater realism in avian risk estimates for PCBs.

**TP009 Evaluation of the effects of ethinylestradiol and chlorpyrifos using an early-life stage Japanese quail toxicity test**

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Birds are sensitive indicators of ecosystem health, but avian toxicity data for many chemicals of environmental relevance are limited. The overall goal of our research project is to develop early-life stage (ELS) avian toxicity tests for rapidly screening chemicals of ecological and regulatory concern. Here, we use our recently developed standardized egg injection protocol to assess the effects of two endocrine disrupters – ethinylestradiol [EE2] and chlorpyrifos [CPF] – on organismal and molecular level end points in ELS Japanese quail (JQ). The chemicals were dissolved in dimethyl sulfoxide and injected into the air cell of JQ embryos prior to incubation at 0, 0.33, 3.33, and 33.3 µg/gegg for EE2, and 0, 0.4, 4, and 40 µg/g egg for CPF. The highest concentration chosen for each test compound was predicted to result in ≤20% mortality. Liver tissue was collected from a sub-set (n=5/dose group) of embryos at mid-incubation (day 9) for subsequent ‘omics and analytical chemistry analyses. The remaining embryos were examined on day 16 (1-2 days prior to hatch) for deformities, growth and health metrics. Mortality was low across all treatments, ranging from 3% for the vehicle group, to 14% and 9% for the high doses of EE2 and CPF, respectively. Exposure to both chemicals led to a significant reduction of embryonic growth and CPF caused an increase in gallbladder size and incidence of deformities of the feet and spinal cord. Analysis of RNAseq data from mid-incubation embryos is currently underway, and samples will be subjected to metabolomics, proteomics and histological analyses as well. The use of avian embryos prior to hatch helps address the need to replace live animal tests with alternative approaches for chemical screening. The co-determination of apical and ‘omics end points will greatly contribute to ecological risk assessments and the development of adverse outcome pathways. This study is part of the EcoToxChip project (www.ecotoxchip.ca).

**TP010 Rodenticide burdens in several species of raptors collected in the Southeastern US**

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Rodenticides have routinely been found in raptor tissues around the world and occasionally these burdens have been associated with mortality events. Monitoring is an important mechanism for discovering burdens that may be associated with lethality or acute toxicity. We began a monitoring project in collaboration with a raptor rehabilitation facility to monitor rodenticides in the livers of raptors that arrived deceased to the facility or were unable to be rehabilitated. We tested for the presence of two first generation rodenticides (diphacinone, chlorophacinone) and three second generation rodenticides (brodifacoum, difenacoum, bromadiolone). Liver samples were ground and extracted with acetonitrile. Concentrated samples were cleaned up with a QUEChERS kit and extracts were analyzed on an LCMS. We analyzed 44 liver samples from 10 species for rodenticide residues. Rodenticide residues were generally low, and no detectable residues of either diphacinone or chlorophacinone occurred in our samples. Residues of brodifacoum were more common while detections of the other SGARs were also fairly low. The number of individuals out of 44 with detectable levels of difenacoum, bromadiolone, and brodifacoum were 1, 1, and 27, respectively. Average recoveries of diphacinone, chlorophacinone, difenacoum, and brodifacoum were all greater than 89% (range: 89.5-102.5%). Recoveries of bromadiolone were consistently lower and averaged 40%. There were certain species with a high rate of detection of brodifacoum. Detections of brodifacoum were > 75% of samples in barred owls, great horned owls and red-shouldered hawks. Red-tailed hawks (40%) and Cooper’s hawks (33%) had lower detections, but also generally smaller samples sizes. The only raptor with more than 3 samples analyzed and no detections was the eastern screech owl. Over 69% of detections of brodifacoum in owl samples were within (or exceeded) a threshold of brodifacoum residue associated with a 10-20% risk of acute toxicity. Rodenticide exposures associated with a risk of acute toxicity appear to be common and warrant further investigation for raptors in the SE United States.

**TP011 Working towards the recovery of the endangered Aplomado Falcon in south Texas: Are persistent organic pollutants a current concern?**

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Addled Eggs from the endangered Northern Aplomado Falcon were collected annually during 2004-2017, from Laguna Atascosa National Wildlife Refuge and Matagorda Island in south Texas. Eggs were analyzed for persistent organic pollutants, including PCBs, PBDEs, and organochlorine pesticides. Eggshells were measured to determine thickness and to correlate with p,p-DDE concentrations. Our hypothesis is that environmental contaminants in Aplomado Falcon eggs have decreased significantly over time and that eggshell thickness values are near pre-DDT measurements. The 60 egg homogenates were extracted, cleaned- up, and analyzed by gas chromatography-mass spectrometry. Eggshell thickness of 137 shells was measured three times around the equator with a Starrett micrometer. Eggshell thickness varied from is 0.206 mm to a maximum of 0.320 mm. Contaminant concentrations were correlated with eggshell thickness. We suspect that eggshell thickness is strongly correlated with p,p'-DDE concentrations, but these analyses are pending. The last reported contaminant concentrations in eggs of Aplomado falcons from south Texas were from 1999 to 2003, with a mean of 821 ng/g ww for p,p'-DDE and 1228 ng/g ww for PCBs. Eggshell thickness decreased slightly with higher contaminant concentrations. This study will provide an update from eggs collected between 2004- 2017. The Northern Aplomado Falcon population in Texas has been steady at over 30 breeding pairs since 2011, but has not grown significantly. This analysis will provide necessary information to determine if contaminants may be impacting population growth and to assess the quality of habitat across the Aplomado Falcon's range.

**TP012 Transcriptional characterization of the effects of polycyclic aromatic compounds (PAC) in double-crested cormorants during embryonic development**

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Double-crested cormorants (*Phalacrocorax auritus*; DCCO) are colonial waterbirds that are more often included in toxicological studies and yet little is known about their transcriptome regulation during embryonic development. Studying how genes change throughout early development is imperative for understanding normal gene control during organogenesis and other developmental mechanisms. Transcriptional developmental profiles can also help in establishing baselines prior to contaminant disruption and can assist in identifying mechanisms of action. The research objectives of this study were 1) to establish the expression profiles of several key genes during DCCO early development and 2) to assess the effects of polycyclic aromatic compounds (PAC) to DCCO egg stage. To do this, 150 DCCO eggs were collected from Mohawk Island in Lake Erie, a relatively uncontaminated reference site. The eggs were artificially incubated for a maximum of 24 days (from fertilized eggs to 4 days prehatching DCCOs). Five embryos were sampled every 4 days for a total of 6 time points. Embryos were sampled as either whole body or specific tissues according to developmental stages. Targeted qPCR analyses displayed that the expression of genes involved in xenobiotic metabolism, antioxidant defense, and thyroid hormone pathways change throughout development. Complementary PAC exposures were conducted in the developing DCCO. DCCO fertilized eggs were exposed *in ovo* to a range of dilutions of coal tar extracts containing PACs. Genes associated with xenobiotic metabolism increased midway through incubation (Day 12); however, the expression levels returned to normal once the birds reached the hatching stage. Taken together, DCCO embryos are more sensitive to PAC exposure during the first X days post-fertilization, but then can metabolize PACs by hatch. Identification of transcriptomic profiles throughout embryonic development can help better relate exposure of metabolizable contaminants with biological effects.

**TP013 An investigation of lethal and non-lethal domoic acid exposure in loons in California**

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Beginning in April 2017, an unusually large number of dead loons were found between Ventura and Los Angeles County in California during systematic beach surveys, and live stranded loons were reported in large numbers at local wildlife rehabilitation centers (Santa Barbara Wildlife Care Network and International Bird Rescue; IBR). Concurrent to this increase in stranded birds, there was a large bloom of *Pseudonitzschia* spp. with domoic acid (DA) toxin production affecting the Santa Barbara Channel area. IBR received 117 live loons between April 4 and May 30, 2017. Loons that entered rehabilitation displayed obtunded to stuporous mentation. Only nine loons showed typical neurologic clinical signs of DA intoxication such as tremors or seizures at admission. Twelve birds (10.3%) died in the first 24 hours despite supportive care. Birds that survived the first few days quickly re-attained waterproof plumage, normal body weight and blood values, and 49 loons (41.9%) were successfully released; however, 56 loons (47.9%) never became releasable due to ongoing abnormal behavior or injuries related to beaching, and were later euthanized. In order to characterize changes to the avian brain after exposure to DA, the brains of six loons euthanized at IBR due to long-term obtundation of up to 85 days duration, were collected for histology, including one bird where the brain grossly appeared atrophied. The California Department of Fish and Wildlife Marine Wildlife and Veterinary Care and Research Center (MWVCRC) performed systematic necropsies on beachcast carcasses (n=14), and samples were collected for histopathologic examination. Because many birds showed typical evidence of DA intoxication on gross necropsy, including congestion in the brain and other major organs, a subset (n=7) were analyzed for DA in collaboration with University of California, Santa Cruz (UCSC). Seven out of 7 birds examined at the MWVCRC tested positive for DA toxin at UCSC. Brains collected during gross necropsy at MWVCRC from beachcast carcasses were also examined for acute exposure to DA. Gross and histological results were reviewed and compared to highlight the differences between lesions due to acute lethal DA exposure and lesions that develop over time subsequent to an acute non-lethal exposure. Currently, little is known about the effects of chronic versus acute DA exposure in seabirds, and our study aims to fill gaps in this body of knowledge.

## Immunoanalytical Technologies: Development and Applications for Environmental Monitoring

**TP014 Fipronil immunoassay: From conception to broad application**

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Phenylpyrazole insecticides such as fipronil have been used as replacements for organophosphates. The wide application of fipronil raises concern about environmental contamination and risk for fish, birds, bees and other non-targeted beings. To mitigate ecological risks routine environmental monitoring could help in timely detection of environmental contamination thus preventing at-risk species from exposure. Two sensitive, rapid tests based on antibodies with sensitivities of 0.58±0.06 and 2.6±0.4 ng/mL were developed. In addition to environmental monitoring, occupational medical surveillance is highly desirable in manufacturing facilities where exposure to chemical is significant. Identification of urinary metabolites of fipronil may allow development of affordable, cheap and rapid procedures for human exposure evaluation. Therefore, we developed a fast and easy approach for synthesis of hydroxy-fipronil, a potential urinary metabolite of fipronil. This standard was used to develop a sensitive analytical LC-MS/MS method with a limit of quantification of 0.4 ng/mL. Fipronil sulfone and hydroxy-fipronil were quantified in urine samples from fipronil treated rats. Fipronil sulfone concentration centered around 20 ng/mL, while the concentration of hydroxy-fipronil was dose-dependent ranging in 10-10000 ng/mL, therefore being a more sensitive marker of fipronil exposure. Immunoassays developed to fipronil also showed cross-reactivity to hydroxy-fipronil. We showed that immunoassay can measure fipronil and its metabolites in samples at levels relevant for human exposure monitoring. With fipronil crisis occurred in Europe in 2017, both the reagents for the immunoassay and a standard for hydroxy-fipronil are in high demand among international profit and non-profit organizations. The reagents are being applied for commercial kits and rapid tests production, instrumental analytical methods development and for monitoring of food and environmental samples. Fipronil immunoassay is also currently used by USGS for field studies.

**TP015 The Application of Monoclonal Antibody-Based Biosensor Analysis for Rapidly Quantifying PAH Concentrations in Porewater at Contaminated Sediment Sites**

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Advances in biosensor technology allow near real-time measurement of contaminants at sub-ppb concentrations in small volume (< 5 mL) aqueous samples providing spatial and temporal resolution of contaminants at scales not easily attainable by traditional analytical methods. Porewater measurements are an ideal application of this technology and have been shown to be important for evaluating the transport, bioavailability and toxicity of PAH in sediments. Correlations between polycyclic aromatic hydrocarbon (PAH) concentrations measured in porewater samples by biosensor (< 1 µg/L to > 600 µg/L) and those measured by GC-MS were excellent and the results can be used to evaluate contaminant distribution, transport and bioavailability. PAH concentrations in porewater samples were measured in the field within minutes after collection to map the spatial distribution of PAHs at contaminated sites undergoing different stages of remediation in the Elizabeth River, VA. Analysis of porewater samples collected at various depths by drive-point piezometer allowed vertical profiling of PAH concentrations within sediments suggesting the input of contaminated groundwater at depth and advective mixing with less contaminated surface water. The ability to measure low concentrations in small volume samples also allowed the collection and analysis of seepage meter output on an hourly basis to demonstrate that tidal pumping and advection were controlling PAH flux to the water column. Average biosensor concentrations were similar to concentrations measured by passive sampling but allowed finer temporal resolution to evaluate the tidal driven mechanisms controlling PAH transport on an hourly scale. The abundant data collected by biosensor allowed a direct comparison of sites under various stages of dredging or capping to evaluate remediation effectiveness and to develop better long-term remediation strategies where advection may play an important role in contaminant transport. At tidally influenced sites, remediation plans need to address advection of surface waters and mixing within contaminated sediments as an important transport mechanism that may hinder remediation success. Contaminant concentrations in porewater should be evaluated as an important factor contributing to sediment toxicity and flux to overlying waters. New antibody-based biosensor technology now provides a more rapid and economical method to make these aqueous phase measurements.

**TP017 Isolation of Ultra-specific Single Domain Antibody for the Detection of Sulfadimethoxine**

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Sulfadimethoxine (SDM) is a long-lasting sulfonamide antimicrobial medication frequently used in veterinary medicine throughout the world. To protect consumers from the risk related to SDM residue in animal derived food, maximum residue limits has been established in many countries. Immunoassays have the advantages of low cost, high sensitive and speed to detect residue in a variety of samples, but most antibodies produced in previous reports cannot distinguish SDM from other sulfonamides due to their similar structure. For this reason, we constructed a single domain antibody (sdAb) library from immunized alpaca and obtained an ultra-specific and sensitive anti-SDM sdAb (H1-17) by a well-designed biopanning strategy. H1-17 could only bind to SDM with negligible cross-reactivity value to other 28 kinds of sulfonamides. In H1-17 based indirect competitive ELISA, the 50% inhibition concentration for SDM was 1.1 ng $mL^{-1}$ in assay buffer and the detection range was from 0.36 to 3.63 ng $mL^{-1}$. To the best of our knowledge, this is the first time that SDM specific antibody was produced. The antibody developed by this technique provide means for developing extremely specific and sensitive analytical assays for measurement of SDM.

## Cradle to Grave Impacts of Nanotechnology

**TP018 Effects of a human food additive, titanium dioxide nanoparticles E171, on *Drosophila melanogaster* – a 20 generation dietary exposure experiment**

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Although a recent human safety reevaluation of food grade E171 $TiO_2$ was performed by a European Food Safety Authority, not many new data were considered and a major lack of multigenerational studies with reproductive endpoints was noted. In this study, we exposed fruit flies to an estimated daily human E171 consumption concentration for 20 generations. Dietary exposure of 20 consecutive generations of *D. melanogaster* to E171 resulted in a change in normal developmental and reproductive dynamics, reduced fecundity after repetitive breeding, increased genotoxicity, and the appearance of aberrant phenotypes. This effect can be seen as one of the classical adaptations of the fruit fly population to a stressor. A pattern that was gradually observed over the 20 generations of flies was shorter developmental time coupled with higher fecundity and egg to adult viability in virgin females, but reduced fecundity at subsequent mating events Marks of adaptive evolution and directional selection were also exhibited. The larval stages were at a higher risk of sustaining damage from E171 as they had a slower elimination rate and therefore accumulated 10 times the quantity of $TiO_2$ compared to adults. This is particularly worrisome, since among the human population, children tend to consume higher daily concentrations of E171 than adults do. The genotoxic effect of E171 was statistically significantly higher in each subsequent generation compared to the previous one. Aberrant phenotypes were likely caused by developmental defects induced by E171 since the phenotypic features were not transferred to any progeny even after 5 generations of consecutive crossbreeding. Therefore, exposure to E171 during early developmental period carries a higher risk of toxicity, and again, in the human population, fetuses and young children would be the most endangered cohort. The fact that the daily human consumption concentration of E171 is able to interfere with and influence fruit fly physiological, ontogenetic, genotoxic, and adaptive processes certainly raises safety concerns.

**TP019 Synthesis And Characterization Of Carbon Nano-particles From Soot And Dates Fruit (Phoenix dactylifera)**

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Carbon nanoparticle was synthesized from lamp soot, petrol soot and diesel soot. Carbon dots was also gotten from (*Phoenix dactylifera*) by microwave-assisted method. The synthesized carbon nanoparticles were characterized using FT-IR and the carbon dots was also characterized using UV – visible spectroscopy and luminescent study. The FT-IR of petrol soot, diesel soot and lamp soot shows IR absorptions, which indicate the functional groups present. The UV – visible spectra of the carbon dots showed absorption peaks. Photoluminescence study of the carbon nanoparticles from petrol soot, diesel soot and lamp soot revealed a characteristic of green and blue luminescence and also with that of carbon dots from date fruit (*phoenix dactylifera*) which showed a characteristic of green luminescence.

**TP020 Microbial Community Responses to Silver Nanoparticles in Freshwater Sediments: A Potential Risk to the Aquatic Ecosystem Health**

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Silver nanoparticles (AgNPs) are among nanomaterials increasingly incorporated into consumer products due to their antimicrobial properties. There are greater concerns about potential antimicrobial effects of AgNPs in aquatic ecosystems following a recent detection of the nanoparticles in treated municipal wastewater. Since treated wastewaters are released into surface waters and metals in aquatic systems eventually settle in sediments, we examined microbial community responses to AgNPs. We treated sediments from a local stream with different concentrations of AgNPs coated with either citrate or polyvinylpyrrolidone. Heterotrophic plate count, microbial enzyme assays, and community level physiological profiling (CLPP) using BIOLOG$^{TM}$ microplates were carried out to determine AgNPs effects on the microbial community. Each type of AgNPs exhibited more than a 60% decrease in microbial growth and glucosidase activity in exposed sediments at 0.431 mg Ag/kg with median inhibition concentrations (IC50) of 0.084 mg Ag/kg compared to the control sediment, but alkaline phosphatase activity was not affected by either AgNPs. CLPP results showed that microbial functional diversity and substrate richness were inhibited by AgNPs affecting carbon source utilization patterns. Principal component analysis demonstrated that microbial metabolic fingerprint patterns of exposed sediments were clearly different from the control sediment, suggesting that the ability of the microbial community to utilize different carbon sources or decompose organic matter decreased in the exposed sediments. The nominal concentrations at which inhibition occurred are below expected AgNPs concentrations in freshwater sediments. This implies that the growing application of AgNPs in consumer products may alter microbial communities in aquatic ecosystems, which may affect the quality of surface waters.

**TP021 Bioavailability of nanoparticles in eastern mosquitofish and freshwater snails following a 9-month exposure in wetland mesocosms**

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Engineered nanomaterials such as metallic nanoparticles (NPs) are ever more widely produced, used and released into surface waters, which may pose a risk to human and ecosystem health. While there is a growing body of research on the toxicity of NPs, risk assessments must account for the bioavailability of NPs at environmentally relevant exposure concentrations and in complex ecosystems. Here, we present results from a study in replicated outdoor wetland mesocosms exploring the bioavailability of metallic NPs in eastern mosquitofish (*Gambusia holbrooki*) and two freshwater pond snails (*Physella acuta* and *Lymnaea* sp.). These aquatic taxa were exposed weekly to pulse doses of either gold (Au) NPs (19 mg per week and primary particle size of 11.8 nm), large or small cerium dioxide ($CeO_2$) NPs (19 mg per week, 185.3 nm and 3.8 nm for large and small $CeO_2$ NPs, respectively), copper hydroxide NPs from Kocide 3000 (3588 mg N mg per week, 118.3 nm), or no NP controls. The Au NP, Kocide 3000 and control groups were also tested with or without weekly nutrient additions (88 mg N, 35 mg P per week) as a covariate. There were 3 mesocosm replicates for each unique treatment group. We quantified the accumulation of NPs in tissues quarterly using ICP-MS over the 9-month experiment (i.e. at 3, 6 and 9 months). During sampling, individuals were euthanized and preserved either immediately or after a depuration period of up to 7 days in clean water. Our results at 9 months indicate that *Physella* accumulated more NPs than *Lymnaea*, and both snails accumulated more NPs than *Gambusia*. The accumulation of copper (after subtracting control background levels) in all taxa was 1-3 orders of magnitude higher than Au or Ce, despite a similar NP dose mass, with *Physella* at the highest relative ratio and *Gambusia* the lowest. In snails, the small $CeO_2$ and Au NPs accumulated equally, both at significantly higher levels than large $CeO_2$ NPs, while in fish the small and large $CeO_2$ NPs and Au NPs all accumulated equally. Fish fully depurated copper NPs and both $CeO_2$ NPs within 12 hours, but were only able to depurate about 68% of the accumulated Au NPs even after 7 days. Preliminary results from the 6-month time point indicate the fish were at the same NP concentration for Au and $CeO_2$ NPs, demonstrating NP accumulation steady state was reached by 6 months. Finally, nutrient additions did not significantly influence long-term NP accumulation or retention.

**TP022 Nano-titanium dioxide enabled products – A review of current status and beyond from a life cycle perspective**

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Titanium dioxide ($TiO_2$) nanoparticles (NPs) are ranked in the top three most produced nanomaterials (NMs) worldwide, with applications as consumer products such as cosmetics, including sunscreens, were among the top usage at 70–80%, followed by plastics (20 %), paints (10–30 %), and cement (1 %). To improve the state of knowledge on the overall sustainability of nano-$TiO_2$ enabled products, the potential impact of nano-$TiO_2$ to human and environmental health should be assessed during their releases into main environmental compartments (water, air, and soil) throughout their lifetime (including manufacture, use, and disposal). Life cycle assessment (LCA) is a highly qualified tool quantifying all relevant environmental impacts of a product from extraction of the raw materials through its production and use up to its final disposal, which has been recognized for systematically evaluating the potential environmental impacts of engineered nanomaterials (ENMs) for the past decades. However, the large variety of existing ENMs and their unique physicochemical properties enhance the complexity and limitations of applying LCA on ENMs specifically. In this review poster, we present an overview of the current LCA performed on nano-$TiO_2$ enabled consumer products, synthesis methods, and identify the needs for future improvements of current progress. Characterization factors (CF) derived for $TiO_2$ NPs for use during LCA are summarized, and potential shortcomings are discussed and recognized. In addition, environmental concentrations and the potential toxicity of $TiO_2$NPs are identified during each of the main routes of NP releases. Based on this analysis, recommendations for LCA to be applied on nano-TiO2 enabled products are provided, which can also extrapolate to other types of NMs for use in LCA.

**TP023 Importance of dissolved silver in the toxicity of silver nanoparticles towards algae**

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Silver nanoparticles (AgNPs) are employed in a variety of consumer products and medical applications due to their antibacterial properties, causing increased discharges of AgNPs into aquatic environment and thereby posing potential hazards to aquatic organisms. Despite the extensive studies on AgNP toxicity, it is still debatable whether the toxicity is mediated by nanoparticle specific effects or by dissolved Ag. This study investigated the toxic effects of citrate-coated AgNPs with different sizes toward a freshwater phytoplankton *Euglena gracilis*, and specifically evaluated the contribution of the released Ag to the toxicity. Our results showed that smaller AgNPs, which dissolved much faster, exhibited greater toxicity to the algae. By using an $Ag^+$ selective probe whose sensing mechanism was based on an aggregation-induced emission process, we visualized the cellular distribution of silver ions using confocal laser scanning microscopy (CLSM). Compared with the 60 nm AgNPs, the 20 nm AgNPs were more prone to solubilize, and more soluble Ag was internalized. However, the internalization of silver ions was dramatically inhibited in the cells exposed to AgNPs or $Ag^+$ by the addition of cysteine. Besides, the average soluble Ag concentrations of both AgNPs at 50% inhibition during exposure time were comparable to those of silver nitrate, and no conclusive evidence of AgNP internalization was observed using transmission electron microscope. Our results therefore strongly suggested that the toxicity of AgNPs toward the algae was driven by release of silver ions.

**TP024 In vivo biotransformation, transportation and biodistribution of silver nanoparticles in Daphnia magna**

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Silver nanoparticles (AgNPs) have been extensively applied in different fields of our daily life and incorporated into a wide variety of consumer products due to their excellent antibacterial properties. The proliferation of applications and products containing NPs will inevitably result in the release of these nanomaterials into the environment, which will interact with aquatic organisms, possibly posing hazards to aquatic ecosystems. Nevertheless, differentiating AgNPs and $Ag^+$ in complicated biological matrix and its dissolution remains a bottleneck in our understanding of AgNPs behavior in living organisms. Here, we for the first time visualized the distribution of AgNPs and $Ag^+$, and conducted time-dependent monitoring of the dissolution processes of AgNPs in *Daphnia magna* based on the novel aggregation induced emission method. Besides, the transportation and biodistribution of AgNPs and $Ag^+$ were monitored. We demonstrated that the ingested AgNPs were dissoluted to $Ag^+$, which was heterogeneously distributed in daphnids, with much higher concentrations in the anterior gut. At dissolution equilibrium, a total of 9.7% and 8.3% of ingested AgNPs was released as $Ag^+$ for 20 nm AgNPs and 60 nm AgNPs, respectively. Further, $Ag^+$ was found to across the gills and enter the daphnids, which may be a potential pathway leading to AgNPs toxicity. Our findings provided fundamental knowledge about the transformation of AgNPs and distribution of $Ag^+$ in daphnids.

**TP025 Silver-selenium interaction and its effect on selenium metabolism studied using the Rainbow trout cell lines RTgutGC and RTL-W1**

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Due to its anti-microbial properties, silver (Ag) has been used for medical and commercial purposes efficiently for several decades. With the increasing demands and use of silver containing products, its long-term adverse effects on human and environmental health is a concern. For instance silver is known to be highly toxic to aquatic species. Moreover, recent research in mammalian cells has clearly indicated detrimental inhibitory effects on selenoenzymes. Selenoproteins are a group of proteins incorporating selenium (Se) as a structural and catalytic co-factor. Due to their role in removal of reactive oxygen species, as well as protection against oxidative damage to cells, they are considered as vital enzymes. Therefore, any disruption of Se homeostasis can be detrimental to organismal health. Our primary objective is to test the inhibitory effects of Ag exposure in the form of ions and nanoparticles on Se homeostasis and selenoprotein function using fish cell lines derived from the Rainbow trout (*Oncorhynchus mykiss*) gut (RTgutGC) and liver (RTL-W1). Therefore, following exposure to non-toxic and moderately toxic (EC10) $AgNO_3$ and Ag nanoparticles doses of exposure, the intracellular Se concentration will be evaluated by ICP-MS. Selenoproteins (Glutathione peroxidase, thioredoxin reductase and selenoprotein P) function will be evaluated by measuring their mRNA levels and enzyme activity post exposure. In conclusion, this study will look at the possible deleterious effects of silver causing disruption of selenium metabolism in fishes and will describe the mechanism behind the toxicity for such compounds.

**TP026 Particle size and surface properties that influence nanoplastics toxicity**

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Environmental contamination with microplastics (plastic < 5 mm) is potentially one of the most long-lasting current pollution problems of our planet. The degradation and disintegration of large microplastics generate nanoplastics, whose accumulation in organisms is of great concern for two main reasons. Firstly, nanoplastics are small enough to potentially cross most biological barriers to environmental contaminants, such as cellular membranes and cell walls, which implies a broad range of potential toxicity targets. Secondly, nanoplastics might present various surface electrical charges that determine the interaction of such particles with biological systems. Thus, while nanoplastics, due to their size, can enter the food web via a higher number of target organisms and taxa compared to larger plastic particles, the reactivity of nanoplastics to membranes, uptake sites and toxicity targets might depend on the nanoplastic surface properties. In order to elucidate the relationship between nanoplastic properties and toxicity we exposed the eukaryote model *Mucor fragilis* to a wide range of concentrations of 12 different polystyrene nanoparticles in liquid culture media. Biomass growth, hyphal reactive oxygen species, and antioxidant capacity were analyzed after 14 days. Ecotoxicological dose-response parameters ($EC_{50}$s, NOEC, LOEC) were determined as a function of particle size (categorical: 25 nm, 50 nm, 100 nm, and 250 nm), surface termination (categorical: COOH, plain, or $NH_3$), nominal mass concentration (up to 1mg $L^{-1}$), particle volume (max ~ $9.6^{-10}$ m³ $L^{-1}$), particle numbers (max ~ $10^{10}$ particles $L^{-1}$), surface area (max 0.01 m² $L^{-1}$) and particle surface Zeta potential (~ -50 mV to ~ +50 mV). While these exposure metrics were good proxies for nanoplastic toxicity, not all were monotonically correlated to the observed responses. For instance, the negatively charged 25 nm particle at 0.1 mg $L^{-1}$ increased fungal biomass. However, at 1 mg $L^{-1}$ all nanoplastic particles triggered cell lysis and biochemical responses that were associated with significantly lower biomass. Therefore, the toxicity of nanoplastics to the chosen eukaryote model could be better explained integrating the multiple exposure metrics into a multivariate space that considers particle size and surface properties. These results are discussed in terms of potential environmental effects of nanoplastics as well as the relevance of choosing the right exposure metrics in nanoparticle toxicology.

**TP027 The characterization of nanoparticle emissions from aerosolized paint dust**

*A. Nored, UAB*

The growing popularity of engineered nanoparticles in manufacturing has raised concerns about the likelihood of exposure to nanoparticles during the end of life phase of nanopaticle infused paint.Weathering and other Destructive situations raise the possibility of both human and environmental exposure to free nanoparticles. This study determines the particle size distribution and total number of particles created using an abrasion technique on paint infused with nanoparticles, and characterizes the dust morphology using scanning electron microscopy(SEM). The results show a particle distribution range from 5-480 nm, with the count median diameter ranging from 43-49nm and a geometric standard deviation of between 1.63-1.67. The SEM images have shown that non-organic materials do both partially and completely escape from organic matter due to abrasion. Exposed particles on the scans are below 100nm. This may be indicative of nanoparticles escaping the parent media.

## Existing, Emerging and New Chemical Contaminants in Changing Polar Environments

### TP028 Does altered contaminant exposure in onshore southern Beaufort Sea polar bears impact immune function relative to those remaining on the sea ice?

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A portion of the subpopulation of southern Beaufort Sea polar bears (*Ursus maritimus*) has been documented spending an extended period of time onshore in the summer as Arctic sea ice cover has waned. While onshore, these polar bears have access to alternative food sources, including carcass remains of subsistence-harvested bowhead whales (*Balaena mysticetus*), fish, and birds. These shifting habitat and diet patterns have the potential to alter the exposures to, and effects of, other environmental stressors such as environmental contaminants. We have previously found differences in levels of certain persistent organic pollutants (POPs) in onshore relative to offshore southern Beaufort Sea polar bears, as well as declines in mercury in this subpopulation linked to shifting diet and condition. Mercury (Hg), and certain POPs, including polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCs), have been linked to altered immune function in marine mammals, and gene transcript profiles from previous blood sampling have suggested immune impairment in southern Beaufort Sea polar bears relative to a neighboring subpopulation. This study evaluates immune responses between onshore and offshore southern Beaufort Sea polar bears, and relationships with tissue Hg (and Se:Hg ratios) and POPs concentrations. Protein mediators called cytokines regulate the initiation, maintenance, and amplification of an immune response. Preliminary findings demonstrated that a commercially available multiplex cytokine panel (Millipore© Canine Cytokine panel) cross-reacted with polar bear cytokines, including important pro- and anti-inflammatory cytokines such as tumor necrosis factor alpha (TNFα), interleukin 10 (IL-10), IL-8, and interferon gamma-induced protein 10 (IP-10). This cytokine panel will be used to measure plasma cytokine concentrations for 52 southern Beaufort Sea polar bears sampled in spring of 2013 and 2014. Relationships among cytokines and hair Hg concentrations, hair Se:Hg ratios, and blood POPs concentrations will be assessed and compared for onshore and offshore bears. Results from this study will support ongoing assessments of the impact of changing Arctic environments on the health of polar bears at both the individual and population level.

### TP029 Halogenated flame retardants and temporal trends in polar bears from the two major subpopulations in Hudson Bay, Canada

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Flame retardants (FRs) are added to commercial products, including plastics, electronics and textiles with halogenated FRs (HFRs), including polybrominated diphenyl ethers (PBDEs), being known contaminants of Arctic concern. Despite these concerns and increasing international regulation on HFRs, there are few reports of temporal trends of PBDEs and non-PBDE HFRs in wildlife from the North American Arctic. For the present investigation PBDE congeners (25) and non-PBDE FRs (24) were measured in the adipose of southern (SHB; 2007–2016) and western (WHB; 1991–2015) Hudson Bay polar bears to examine spatial and temporal trends. Most of the non-PBDE FRs, with the exception of hexabromocyclododecane (HBCDD) and polybromobiphenyls (BB)-101 and BB-153, were largely below detection limits in both subpopulations. HBCDD was detected consistently in the WHB from 2001–2011 and in the SHB from SHB from 2007–2012 (linear regressions were not significant, $p > 0.10$), but was below detection limits outside of these ranges. BDE-209 was detected infrequently in the WHB from 2003-2012 and in the SHB from 2008–2013; like HBCDD, it has not been detected in recent years (unsuitable for temporal analysis). Of the consistently detected compounds, BDE-47, BB-153 and BDE-153 had the greatest concentrations in both subpopulations. BB-153 was decreasing significantly in the WHB (-1.8%/y; half-life = 38 y) and may be decreasing in the SHB, however the linear regression was not significant (-6.3%/y, $p = 0.22$). The $Sum_4$PBDE (BDE-47, -99, -100, -153) concentrations were increasing relatively rapidly in the WHB (9.1%/y) with a doubling time of 7.6 y, however this relationship was also insignificant in the SHB (1.4%/y; $p = 0.37$). Only BDE-99 yielded a significant linear relationship in the SHB, decreasing at -3.3%/y with a half-life of ~21 y. In contrast, BDE-47, -99, -100 and -153 all increased linearly in the WHB at rates of 7.2 to 9.5%/y with doubling times of 7.3 to 10 y. Further analysis of the WHB trends using piecewise regressions showed that concentrations of $Sum_4$PBDE, BDE-47, -99 and -100 increased rapidly (15–16%/y) from 1991 to their breakpoints (~2000-2003), where trends slowed and/or remained relatively constant (-0.0042–6.0%/y) from 2003 to 2015-2016. The breakpoints for these congeners from the pentabromo-DE technical mixtures correspond well with voluntary national reductions in use and North American bans, and agree with observations in other arctic marine mammals.

### TP030 Subcellular distribution of trace elements in polar bears (*Ursus maritimus*) livers

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Polar bears (*Ursus maritimus*) are endangered marine mammals of the Arctic that occupy top trophic positions. The melting of sea ice in response to global climate change is affecting its natural habitat and reducing its hunting territory. In addition, its apical position in the food chain increases its susceptibility to be exposed to contaminants by bioaccumulation, such as mercury (Hg) or others trace elements. This exposure may affect its ability to cope with environmental stressors or ultimately decrease survival. In this context, we aim to evaluate if the intracellular handling of metals – including rare earth elements – in polar bear hepatocytes represents a toxicological risk. For this purpose, we measured arsenic (As), silver (Ag), cadmium (Cd), lanthanum (La), neodymium (Nd), total mercury (Hg) and thallium (Tl) concentrations in the livers of 9 polar bears collected in Greenland and explored how these elements were distributed at the subcellular level. The livers were subjected to a subcellular partitioning procedure using differential centrifugation, NaOH digestion, and heat denaturation steps, that separate liver cells into five subcellular fractions. These fractions were categorized into putative metal-sensitive (heat-sensitive fractions, mitochondria, microsomes+lysosomes) and detoxified metal compartments (heat-stable proteins, granule-like structure). The presence of trace elements in the metal-sensitive fractions may reflect a toxicological risk for the polar bears. The fractions were analyzed for trace elements by ICP-MS. Results demonstrate that the heat-stable protein fraction is the main fraction involved in detoxification for the six trace elements studied, with up to 60% of the total Cd and Ag accumulated therein. The trace elements were also found in the metal-sensitive fractions, predominantly in mitochondria. Indeed, for all metals analyzed, at least 15% of the total metal accumulated was associated with the mitochondria. The proportions of both As and Tl in the metal-sensitive compartment approached ~60%. Accumulation of As in the metal-sensitive compartment exceeded accumulation in detoxified fractions for certain individuals, whereas this was the case for Tl and Nd for all individuals. These results indicate that polar bears are able to detoxify these trace elements, but this detoxification is not sufficient to prevent their association with physiologically sensitive sites, which could compromise polar bear health.

**TP031 Can faeces serve a proxy for the polar bear's contaminant body burden?**

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Over the last decades, evidence has accrued showing that Arctic ecosystems are exposed to contaminants, such as persistent organic pollutants (POPs) and mercury. These contaminants are of concern because of their persistence, toxicity, and bioaccumulation rate. Polar bears, as apex predators, can biomagnify such contaminants and are a good indicator of Northern environmental pollution. Several studies have quantified contaminant body burden in polar bears, but these studies have relied on invasive methods, including collection of fat, liver, teeth, or kidneys. The novelty of this project centres on using a non-invasive and robust monitoring method using polar bear faeces to quantify contaminant loads. The objective of this work is to establish a database of contaminant concentrations in bear tissues to predict human exposure and risks for local indigenous people (e.g., via traditional subsistence hunting). Preliminary data show high levels of total mercury and metals in polar bear's faeces. Complementary analytical analyses (e.g., PCBs, PBDEs, PAHs, organic pesticides, and MeHg) are in progress. Our study will facilitate contaminants monitoring in polar bears while minimizing contact between humans and bears.

**TP032 Subpopulation- and Compound-Specific Temporal Changes of Perfluoroalkyl Acids and Precursors in Polar Bears From Hudson Bay**

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Perfluoroalkyl carboxylic acids (PFCAs) and sulfonates (PFSAs) in the environment include perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) as well as their precursors. PFOS, PFOA and perfluorohexane sulfonate (PFHxS) have been increasingly regulated and phased-out, and have been replaced by new PFAAs such as shorter-chain analogues (e.g. perfluorobutane sulfonate (PFBS) and carboxylic acid (PFBA)), perfluorooctane-1-ethylcyclohexyl sulfonate (PFEtCHxS) and other precursors of perfluoroalkyl acids (PFAAs). Hudson Bay is an Arctic contaminant and climate change hot spot and polar bears (*Ursus maritimus*) are a top marine-feeding predator. In the present study, compound-specific temporal trends (for all years from 2007 to 2016) were examined in the liver tissue of Hudson Bay polar bears (southern (SHB) and western (WHB) subpopulations). For temporal statistical analysis, compounds were used if detected in >80% of total the samples (n=225) and medians were used to minimize outlier effects. For the SHB bears, PFSAs gradually decreased over time but only the PFEtCHxS and PFOS decreases were significant ($p< 0.05$) with changes of -6.3% and -25% per year, respectively. Although not significant, the PFOS precursor perfluorooctane sulfonamide (FOSA) increased by 1.4% per year. PFBS and the perfluorobutane sulfonamide (FBSA) were quantifiable in only 43% and 18%, respectively, of the samples. PFUdA, PFDoA and PFTrDA decreased 13-14% per year, but the shorter-chain PFOA and PFNA increased by 0.5% and 7.1% per year. PFBA was quantifiable in only 36% of the samples. Different from SHB bears, PFOS in WHB bears remained unchanged, but PFEtCHxS and PFHxS both increased by 5%, and FOSA increased by 18% per year. Similar to the SHB bears, PFBS and the FBSA precursor were both quantifiable in only 25% of the samples. PFUdA, PFDoA and PFTrDA in the WHB bears appeared to decrease 11-15% per year, and in contrast to SHB bears, the shorter-chain PFOA and PFNA appeared to show greater increases of 8.4% and 24% per year. Like the SHB bears, PFBA was quantifiable in only 34% of the WHB samples. These results clearly showed temporal changes or a lack of change in the concentrations of PFAAs and/or their precursors, which differed between bears of the two subpopulations. We are currently examining the source, dietary, etc. factors that may explain these temporal changes in polar bears.

**TP033 Comparison of trends of perfluoroalkyl substances (PFASs) in ringed seals and in seawater across the Canadian Arctic**

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The ringed seal (*Phoca hispida)* is the most abundant Arctic pinniped with a circumpolar distribution and a key biomonitoring species for the evaluation of contaminants. Early temporal trend studies on perfluoroalkyl substances (PFASs) in liver of ringed seals at two locations in the Canadian Arctic, showed C9–C15 perfluorocarboxylates (PFCAs) had increasing levels during the period 1998-2005 while perfluorooctane sulfonate (PFOS) achieved maximum concentrations in 1998-2000 and declined from 2000 to 2005. Ocean transport of PFASs was proposed as the main route of deliver of PFASs to the arctic marine environment, however, the rapid response to phase out of PFOS suggested the importance of an atmospheric pathway as well. The objective of this study was to compare temporal trend data for PFASs in ringed seals in several widely separate locations in the Canadian Arctic over a 15 to 20 yr period, as well as newly available time trends in ocean waters from the central Canadian archipelago, and with trends in atmospheric concentrations. Ringed seal samples were obtained from subsistence harvesting in four regions of the Inuit Nunangat: Sachs Harbour (NWT), Resolute and Arviat (Nunavut) as well as in Nain (Labrador). Seawater (1L) was collected in Lancaster Sound, near Resolute Bay, between 2005 and 2017 at depths of 10-100m. Seal liver and seawater were analyzed for PFASs by LC-MS/MS. Highest total PFASs (PFOS + C8–C15 PFCAs) in ringed seals over the period 2011-2016 were observed in the more southern locations, Arviat (52 ng/g ww) and Nain (41 ng/g ww), compared to more northern (Sachs Harbour; 30 ng/g; Resolute 24 ng/g). Lowest concentrations of PFOS were generally observed during the period 2005-2010, following a decline from higher levels in the period 1998-2005. Since 2011 PFOS concentrations in liver have increased at Resolute and Arviat by about 2-fold. The trends for PFCAs generally show a similar temporal pattern as PFOS with indications of recent increases particularly at Arviat. In seawater collected near Resolute since 2005, PFOS has declined significantly with an overall disappearance half-life of 4.4 yr. PFOA showed no trend between 2005 and 2017 in seawater. The increase in PFOS in seals is also observed in atmospheric measurements in the Canadian Arctic where PFOS had a doubling time of 2.9 years between 2006 and 2014. Thus trends of PFOS concentrations in ringed seals appear to reflect atmospheric rather than oceanic trends in the Canadian arctic.

**TP034 Engaging Indigenous communities in contaminants research on wildlife: Educational workshops on ringed seal health conducted in Inuit Nunangat**

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The ringed seal is a species of high cultural, economic and nutritional importance for northerners living in Inuit Nunangat (Inuit regions of Canada). Scientists conducting studies of the spatial and temporal trends of legacy and emerging/new contaminants in ringed seals from the Canadian Arctic work towards better understanding the ecology and health of this species, often in collaboration with Inuit community members. This project addresses a shared interest among Inuit and scientific researchers in enhancing communications and northern community capacity building in relation to contaminants research. It engages scientific researchers, Inuit youth, elders, and community members in learning about ringed seals from both Inuit Qaujimajatuqangit (Inuit traditional knowledge) and scientific perspectives through educational workshops. Since 2016, we conducted three educational workshops in collaboration with local schools and Hunters and Trappers Organizations. The main goals of these workshop were to (1) allow scientists working on contaminants in ringed seals to share information about their work with northern residents (with a focus on Inuit youth in particular), (2) provide an opportunity for Inuit elders to share their knowledge with students and researchers in seal ecology and traditional methods for hunting, preparing seals and identifying abnormalities in harvested animals, (3) increase the engagement and interest of northern students and community members in contaminants research, and (4) identify best practices and lessons learned for engaging and communicating with Inuit youth and elders as part of contaminants research. The experience and insights gained from this innovative work can be helpful to scientific researchers, educators, and community members interested in Indigenous engagement in contaminants research on wildlife, and the development of educational opportunities linking Indigenous knowledge and contaminants research.

**TP035 Screening-level risk assessment of methylmercury for non-anadromous Arctic char (*Salvelinus alpinus*)**

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Arctic char (*Salvelinus alpinus*) is an ecologically and culturally important species of fish distributed across northern regions of the Holarctic. Anadromous forms of Arctic char, those that migrate to and from the sea, are typically lower in mercury concentrations than non-anadromous forms that are restricted to lakes and rivers. Using data from the literature and our own analyses, we performed a screening-level risk assessment of methylmercury for non-anadromous Arctic char. Our assessment included more than 1500 fish distributed across 81 sites. Site-specific mean total mercury concentrations in non-anadromous Arctic char muscle varied considerably from 0.01 to 1.13 µg/g wet weight, with 21% (17 of 81 sites) meeting or exceeding a threshold-effect level in fish of 0.33 µg/g wet weight, and 14% (11 of 81 sites) meeting or exceeding 0.5 µg/g wet weight. Of the sites in exceedance of 0.33 µg/g wet weight, seven were located in Greenland and ten in Canada (Labrador, Nunavut, Yukon). Maximum total mercury concentrations exceeded 0.33 µg/g wet weight at 55% of sites (40 of the 73 sites with available maximum total Hg values), and exceeded 0.5 µg/g wet weight at 28% (20 of 73 sites). Collectively, these results indicate that certain populations of non-anadromous Arctic char may be at risk for methylmercury toxicity. Furthermore, the most contaminated individuals in more than half of the populations may also be at risk. Though the approach used in this study provides a simple statistical assessment of methylmercury risk to non-anadromous Arctic char, it does not indicate actual effects. We highlight the need for laboratory and field studies which evaluate the potential toxic effects of methylmercury in non-anadromous Arctic char, as well as those that aid in the development of a methylmercury toxic-effect threshold specific to this species of fish.

**TP036 Spatial and long-term trends in persistent organic contaminants and mercury in lake trout and burbot from Great Slave Lake, the Northwest Territories**

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Great Slave Lake, located in Canada's Northwest Territories, is a large (28,568 km$^2$), deep (mean depth 69 m, maximum depth 614 m) lake and can be subdivided into two major ecoregions. The West Basin is relatively shallow, productive, and profoundly influenced by Slave River flow which originates in the more developed regions to the south; the East Arm is substantially deeper, lower in productivity and with small riverine inputs from the Canadian Shield. Long-range atmospheric sources are inferred to be the primary contaminant source to the East Arm while the West Basin also receives Slave River sources. The region is undergoing pronounced warming trends which appear to be affecting productivity and may affect mercury methylation rates and various aspects of fish biology. Here we report on our long-term (1993-2017) investigations of spatial and temporal trends in mercury and persistent organic contaminant concentrations (POCs) in lake trout (*Salvelinus namaycush*) and burbot (*Lota lota*). Overall, mercury concentrations were relatively low in lake trout and slightly higher in West Basin (0.21 ±0.08 µg/g) than East Arm (0.17± 0.10 µg) fish; mercury concentrations were similarly low in burbot (0.16±0.07 µg/g West Basin; 0.14±0.06 µg/g East Arm). Relatively low mercury concentrations in comparison to smaller lakes in the region is attributed to large lake effects including cold waters throughout most of the year and weak watershed inputs and their influences on methyl mercury production. While mercury concentrations exhibited a trend of increase up to ca. 2012, this trend has weakened in recent years as has been observed elsewhere in the north. Legacy POCs concentrations tended to be slightly higher in East Arm than West Basin fish, e.g., considering ΣPCBs, concentrations averaged 12 ± 12 ng/g for West Basin lake trout fillet versus 19 ± 18 ng/g for East Arm trout and 85 ± 55 ng/g for West Basin burbot liver versus 97 ± 62 ng/g for East Arm fish) suggesting that anthropogenic influences from the south were not exerting detectable contaminant effects on West Basin fish. POCs concentrations were in the middle of the range for lakes investigated in northern Canada and the Laurentian Great Lakes. ΣChlordane, ΣDDT, ΣHCH and ΣPCB concentrations have declined significantly ($p < 0.05$) in West Basin lake trout and burbot while significant trends have been detected only for ΣDDT and ΣHCH for East Arm fish. East Arm fish may be slower to show trends in POCs decline because of the longer residence time of its water and the lower concentrations of particulates which may allow POCs to remain in the water column for a longer period of time than in the West Basin. ΣPBDE and ΣPFCA concentrations were low; trend investigations are underway. Overall, mercury and POCs concentrations are low in Great Slave Lake fish; mercury trends are of particular interest as warming trends continue.

**TP037 Investigating the presence and persistence of volatile methylsiloxanes in Arctic sediments**

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Volatile methylsiloxanes (VMS) are a group of organosilicon compounds that are used as carriers in personal care products and in the production of silicone polymers. Concerns have been raised about the persistence of VMS in aquatic environments due to their long residence times in sediments.[1] Reported measurements of the partition ratio between organic carbon and water ($K_{OC}$), the enthalpy of sorption ($\Delta H_{OC}$) and the enthalpy of phase change between octanol and water ($\Delta H_{OW}$) of VMS are subject to large variability, resulting in large differences in calculations of persistence and bioaccumulation. These differences may be as large as 1100-days difference in overall persistence and 1 order of magnitude in concentrations in biota. We collected sediment samples from three areas in the Arctic (Svalbard, Greenland and Canada), and wastewater samples from Svalbard. The samples were analyzed for 3 linear VMS (lVMS): $L_4$, $L_5$, $L_6$, and 3 cyclic VMS (cVMS): $D_4$, $D_5$, $D_6$. We used the measured concentrations of $D_5$ in the wastewater samples to predict concentrations in the sediment and we compared them to measured values. For our modeling calculations, we evaluated two different scenarios: (1) we used the $K_{OC}$ measurements of Kozerski et al. and the $\Delta H_{OW}$ of Xu and Kropscott, (2) we used the $K_{OC}$ and $\Delta H_{OW}$ measurements of Panagopoulos et al.[2-3] The highest concentrations measured for all VMS were observed in Greenland. The predicted concentrations for $D_5$ in scenario 2 were in good agreement with the measured concentrations, whereas in scenario 1, they were almost 2 orders of magnitude lower. Based on our sensitivity analysis, the required changes for the predicted concentrations of scenario 1 to reach the concentrations of scenario 2 ranged from 2 orders of magnitude (emissions rates) to 8 orders of magnitude (density of sediment particles). Such large discrepancies indicate that the differences in the predicted concentrations in the two scenarios are more likely to be attributed to $K_{OC}$ and $\Delta H_{OW}$ / $\Delta H_{OC}$ than in environmental parameters or emission rates.

**TP038 Modeling of bromine chemistry and the boundary-layer depletion of ozone and mercury across the springtime Arctic**

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Gas-phase bromine radical chemistry is the main driver for the frequent and concurrent depletion of ozone and mercury (Hg) in the polar boundary layer during the spring. Sea ice and its overlying snow cover are broadly understood as the key elements in the production of reactive bromine in polar spring. However, a full characterization remains unsettled on how physicochemical states of snow and ice influence the release of bromine into the atmosphere. Uncertainties in the kinetics and reaction mechanisms of Hg redox chemistry add further complexity to accurately assessing the behavior of Hg during its depletion from air. Three-dimensional (3-D) models, developed to simulate the impact of bromine chemistry on Hg oxidation at both global and arctic-basin regional scales, have generally relied upon indirect representations of the sources, sinks and photochemical transformation of bromine radical species in the polar atmosphere. Within Environment and Climate Change Canada's air-quality model, GEM-MACH, we have developed a process-oriented representation for the coupled bromine-ozone-Hg chemistry and the exchange of bromine, ozone and Hg species between air and snow/ice surface. The model is run at 15-km horizontal resolution in a limited-area domain of the Arctic and is capable of capturing the evolution of high BrO column densities associated with synoptic weather disturbances during polar sunrise as can be seen from satellite. The concurrent depletion of ozone and Hg is simulated by consistent model formulations, where the release of reactive bromine from the frozen surfaces is facilitated by the presence of ozone in air. The concentrations of ozone and speciated Hg measured in the surface air and the vertical column densities of BrO retrieved from ground stations and buoys floating on the ice-covered ocean also allow us to evaluate the model simulations at fine temporal scales even though limited in spatial coverage. Our model framework for simulating the reactive bromine release from the snow/ice cover is found to work reasonably well for the representation of ozone and Hg depletion events across the Arctic region over synoptic and seasonal time scales. The deposition of oxidized Hg from the atmosphere is found to be enhanced particularly under the disturbed weather conditions, as a result of the vertical and horizontal inflow of ozone and gaseous elemental Hg and their chemical conversion in the polar boundary layer.

## Translating Environmental Science into Improved Outcomes and Policy

**TP039 Challenges in Development and Application of Dissolved Oxygen Criteria in Estuarine and Marine Waters**

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Dissolved oxygen (DO) dynamics in coastal and estuarine systems are complex, often depending on temperature, nutrient availability, photosynthesis, and hydrological characteristics of a distinct waterbody. Advances in continuous monitoring technology are better able to capture the intricacies of DO dynamics but provide some uncertainties for developing appropriate DO criteria for marine and estuarine waters. The Environmental Protection Agency (EPA) released guidance for developing DO criteria for the area between Cape Cod, MA and Cape Hatteras, NC. The methodology-- *Ambient Aquatic Life Water Quality Criteria for Dissolved Oxygen (Saltwater): Cape Cod to Cape Hatteras (EPA 2000)* -- describes calculation of endpoints protective of survival, growth, and larval recruitment in aquatic organisms. The results of continuous exposure laboratory toxicity tests shown in EPA (2000) along with more recent data are used to derive the two most commonly used endpoints. The methodology has been subsequently applied, with some modifications, to estuaries and coastal areas around the country. Case studies from several states demonstrate the use of the original guidance, updates to include recent data, potential for use of alternative endpoints such as behavioral studies and field observations, and site-specific ecological or hydrological data that help to remove some uncertainty of applying laboratory endpoints to derive site- and state- specific DO criteria.

**TP040 Is My Cannabis Product Safe? A Closer Look at the Legal Limits for Residual Solvents**

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Recreational cannabis legalization has been growing in popularity over recent years, bringing with it a variety of cannabis-infused products such as vaporizer pens, edibles, and ointments. Such products are made by extracting cannabinoids from cannabis plant material using solvents such as ethanol, butane, and propane. To protect consumer safety, several states have implemented action levels as standards that define the legal limits of residual solvents permitted in cannabis-infused goods. In this presentation, the action levels of California, Colorado, Massachusetts, Oregon, and Washington are reviewed to provide insight to the development, consistency, and applicability of residual solvent limits for cannabis-infused goods across the country. While action levels established by most states were consistent with those adopted by international organizations for residual solvents in pharmaceuticals and based on conservative, health-based approaches, they appeared to be applicable primarily to oral exposures. California was the only state with an additional set of action levels established as air concentrations for inhalable goods; however, the inhalation action levels were based on occupational exposure limits and must be converted to units consistent with those required for laboratory testing (e.g., ug/g) in order to be meaningful. Although action levels established for cannabis-infused goods meant for ingestion generally appear to be protective of human health, consideration must be given to a broader picture of toxicity and exposure in order to reduce potential health impacts associated with other anticipated routes of exposure. Future refinements of residual solvent action levels should consider toxicity via inhalation and dermal exposure, receptors of interest, the magnitude of consumer exposure via consumption of cannabis, and the feasibility of determining regulatory compliance when action levels are expressed in units of measure which are not directly comparable to laboratory results.

**TP041 Translational environmental science: Improving outcomes and policy**

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Translational science has rapidly become an established discipline in medical fields, in which it is described as the non-linear, multi-directional progression of science from "bench" (i.e., basic research) to "bed-side" (i.e., improved outcomes) to "community" (i.e., public understanding). Improved translation from research results to regulatory and practical applications as well as community understanding is demonstrably needed in the multi-disciplinary environmental sciences. Properly understood, one role of translation in the process of developing regulation is the clear separation of policy (societal objectives) considerations from scientific considerations. For example, the use of assumptions, protective safety factors, inter-species extrapolations, and other outcome objectives are part of the translational process and need to be clearly recognized as such. Risk assessment should be understood as a translational tool, separating scientific information from risk management. The historical development of environmental regulation illustrates both successful and less-than-successful translations, and provides guidance for improving the processes of scientific assessment and verification, translation from science to practical outcomes and effective regulation, and improved understanding of the promulgated product.

## Human Exposure to Emerging Environmental Contaminants

**TP042 From biomonitoring to PBPK/PD modeling of Bisphenol A and its analogues: The mixture approach**

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Endocrine-disrupting chemicals are substances of special concern due to their adverse effects for the human health. Among them, bisphenol A (BPA), a chemical widely used for coating in food and drink cans, has attracted most of the attention, being banned in plastic baby bottles in a number of countries. Consequently, BPA has started to be replaced by a number of alternatives, such as bisphenol S (BPS) or bisphenol F (BPF). The present study was aimed at assessing the exposure to bisphenols, the biodistribution in the human body, and finally estimating the potential risk of BPA alternatives. Firstly, the concentrations of these 3 chemicals were determined in foodstuffs collected in different locations, with a special emphasis on the analysis in canned foods. These results were used to assess the cumulative exposure to bisphenols. Human exposure was performed both deterministically and probabilistically taking into account recent food consumption data from the adult population of Spain, by means of the Monte Carlo Risk Assessment (MCRA) software. In parallel, up to 25 people were recruited to build a cohort. A duplicate diet calculated for two days of feeding was purchased. One food basket was provided to each participant, while its duplicate was transported to the lab for analysis. Participants collected 100mL of urine from each micturition along the study, while blood samples were extracted the first day at 8h (fast), 11h, 14h and 17h to further determine the contents of BPA, BPS and BPF. Subsequently, a generic PBPK/PD model will be adjusted to bisphenolic compounds to estimate the concentrations in, at least, 7 body compartments (blood, muscle, skin, richly perfused, adipose, kidney, and liver), in addition to urine and plasma. The results from the duplicate diet study of the present study will be used as model input data, while data from the biomonitoring study of a single adult population will be used for validation purposes. These findings will help elucidate the potential risk of BPA alternatives through food consumption, under a cumulative/aggregated rather than single exposure.

**TP043 Urinary concentrations of environmental phenols and their association with type 2 diabetes in a population in Jeddah, Saudi Arabia**

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A fewepidemiologic studies suggest that exposure to bisphenol A (BPA) is associated with type 2 diabetes mellitus (T2DM). However, little is known about association between other phenolic endocrine disrupting chemicals (EDCs) and T2DM.In this case-control study,we measured urinary concentrations of 23 phenolic EDCs in 101 individuals from Jeddah, Saudi Arabia, to examine the association of parabens, antimicrobials, bisphenols, benzophenones and bisphenol A diglycidyl ethers with T2DM. Urine samples were collected from 54 T2DM cases and 47 non-diabetic individuals (controls), aged 28-68 years old, during 2015-2016. Unconditional logistic regression was performed to estimate odd ratios (ORs) for the association between diabetes and EDC exposures after adjusting for confounders including age, gender, nationality, smoking status and occupation. Age from 40-59 years (OR 5.56, 95% CI 2.20-14.0) and smoking status (OR 2.92, 95% CI 1.25-6.79) showed significant positive associations with T2DM. After adjusting for potential confounders, we found that T2DM cases had high urinary levels of parabens (i.e., methyl- (MeP), ethyl- (EtP), propyl- (PrP) and 4-hydroxy benzoic acid (4-HB)), bisphenols (i.e., bisphenols A (BPA) and F (BPF)), and benzophenone (i.e., 4-hydroxybenzophenone (4-OH-BP)) relative to the controls. Individuals in the 4th quartile for urinary concentrations of MeP, EtP, PrP, 4-HB and BPF and in the 3$^{rd}$ quartile for BPA and 4-OH-BP showed over a 6-fold increase in the odds of having diabetes compared with those in the first quartile. Overall, our study shows that urinary levels of multiple phenolic EDCs were associated with increased risk for diabetes. Further prospective studies are required to verify these associations.

**TP044 Parabens in human urine from several Asian countries, Greece, and the United States**

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Parabens, esters of para-hydroxybenzoic acid, are commonly used as antimicrobial preservatives in cosmetics and personal care products. Although several studies report exposure of humans to parabens in Western countries, little is known about the exposure of humans to parabens in Asian countries. In this study, we determined concentrations of six parabens in spot urine samples collected from nine countries and estimated daily intakes (DI) and potential health risks of parabens. Ethyl-paraben, methyl-paraben, and propyl-paraben were detected frequently at 100, 98.0, and 80.3%, respectively, with representative median concentrations of 0.68, 7.02, and 1.21 ng/mL, respectively, for all nine countries. Urine samples from females (total median concentration: 32.3 ng/mL) contained significantly higher concentrations of parabens than those from males (5.46 ng/mL). Urine samples from Korea (total median paraben concentration: 227 ng/mL) had the highest concentrations, which were one to two orders of magnitude higher than those found in other countries (3.67–29.1 ng/mL). The estimated DI of parabens (on the basis of concentrations measured in urine) varied widely, and several samples had propyl-paraben exposures above the acceptable DI. Our results suggest that paraben exposure is ubiquitous in Asian countries, and further assessment of potential health risk of these chemicals is needed.

**TP045 PBDE, PCB and OCP concentration in breast milk from three Colombian cities (Bogota, Cartagena and Medellin)**

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Concentrations of seven congeners of polybrominated diphenyl ethers (PBDEs) were reported for first time from Colombia in breast milk (BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154 and BDE-183). Thirty-seven polychlorinated biphenyls (PCBs) and fourteen organochlorine pesticides (OCP) were also analyzed in 60 human breast milk samples from volunteering mothers collected in 2015 in three important cities from Colombia. Most of the investigated PBDE congeners were detectable in the breast milk, ranging in concentrations from 0.13 to 0.78 $ng.g^{-1}$ lipid wt. BDE-47 congener had the highest concentration among the samples. Overall mean concentrations of OCP oscillated between 0.043 to 4,709,478 $ng.g^{-1}$ lipid wt. Endosulfan II was greatest concentrations among OCPs found in the matrix analyzed. On average, Cartagena was the city with the highest concentration of PBDEs 0.894 $ng.g^{-1}$ lipid wt, followed by Bogota 0,353 $ng.g^{-1}$ lipid wt and Medellin 0,338 $ng.g^{-1}$ lipid wt. Overall mean concentrations of pesticides in the cities was strongly correlated with the mean of PBDEs. OPC concentrations, on overall were 54,0631 $ng.g^{-1}$ lipid wt for Cartagena, 364,038 $ng.g^{-1}$ lipid wt and 247,742 $ng.g^{-1}$ lipid wt for Bogota and Medellin respectively. This results could be related to the city location and diet. Cartagena is the only city located on the Caribbean Sea, for instance the population consume fish more often than the population of Medellin and Bogota.

**TP046 Determination of Carbazole and Halogenated Carbazoles in Human Blood Samples using GC-MS/MS**

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Carbazole and halogenated carbazoles have been widely detected and reported throughout the environment in soil, river deposits, and lake sediments. Human exposure to these compounds may occur through food, water, and air; bioaccumulation in the body might be evaluated by measuring the levels in blood. This paper reports the method development and validation for the analysis of carbazole and 11 halogenated carbazoles in human blood samples. A small sample size of 100 microliters of human blood or serum was employed for the analysis. The samples were prepared through salting-out liquid-liquid extraction (LLE) by using ethyl acetate/hexane (1:4, v/v) as the extraction solvent and aqueous $MgSO_4$ (38.5 wt%) as the salting-out regent, respectively. The extracts were then concentrated to near dryness under nitrogen flow, and re-dissolved in toluene. Sample analysis was performed on gas-chromatography (GC) coupled with tandem mass spectrometer (MS/MS) in an electron impact mode. The developed method has been validated and demonstrated low detection limits in the range of 0.02 – 0.27 ng/mL. The intra-day accuracy ranged from 81.2% to 125%, and the inter-day accuracy from 91.0% to 117%. The intra- and inter-day precisions, calculated by relative standard deviation (RSD), were in the ranges of 1.0–16.0% and 1.8–16.4%, respectively. The developed method was applied to the analysis of 50 human serum samples collected from pregnancy women in Southern California in 2012 – 2013. Low concentration of carbazole (below quantitation limit) was measured in 18 samples, while none of the halogenated carbazoles was detected in any of the samples.

**TP047 Using high-resolution gas and liquid chromatography to determine anthropogenic chemical exposure in youths of the Czech Republic**

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Human exposure to chemical contaminants has been a topic receiving greater interest over the last decade with projects such as NHANES in the United States and the HBM4EU in Europe. Both these and many other studies aim to determine a wide range of human contaminants. However, every year hundreds of new compounds enter the market and thus the number of compounds we are exposed to is growing. High-resolution mass spectrometry (HRMS) is becoming more and more affordable as a tool for accurate mass determination of contaminants. Full scan acquisition by HRMS provides detailed information on a wide range of compounds while potentially less sensitive than target MS the wealth of compounds determined is far superior. By using both GC and LC HRMS in various ionization modes, it is possible to identify a massive amount of information on chemical exposure. The aim of this project is to provide quantitative information on exposure of 200 youth samples (< 18 years of age) along with suspect and non-target statistical analysis. To do this a standard mixture of >800 compounds of interest including PFAS, PCBs, flame-retardants, personal care products, plasticizers and drugs was produced. From this mixture LC and GC calibration solutions were made for quantification. Two hundred serum samples of children were used in this study; they were collected by the University Hospital for oncology screening and remaining material released to this project with ethical approval from the University Ethics committee. This study does not aim to provide insights into specific cancers but rather to indicate the overall exposure of youths in the Czech Republic. A simple extraction technique to minimize compound loss was developed with 60-70% recovery of most compounds. Samples were extracted and ran on GC-Orbitrap MS in EI and NCI and by LC-Orbitrap Fusion in ESI positive and negative. The mass range used on both instruments was 70-1000amu. For the LC fusion, its dual detectors allowed for low resolution MS2 on target compounds while DIA on the top 10 most abundant ions was used for unknowns. In GC-EI-Orbitrap some 200 compounds were determined in samples >LOD while non-target detection of compounds using various deconvolution methods indicated that 1000s of additional compounds were present. In LC-Orbitrap, many 100s of compounds were also identified with many more unknowns. Suspect screening of compounds by both LC and GC indicate many compounds for further investigation.

**TP048 First measurements of the urban chemical exposome in a mega city**

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The chemical exposome is a comprehensive description of the chemicals to which one person is exposed from conception onward and could be the chemical equivalent of the environmental microbiome. This project aims to explore the urban chemical exposome of people living in a city of over 7 million people (Santiago, Chile). Samples (vapor phase atmospheric samples) were collected using personal passive samplers (ready-to-use silicon wristbands from My Exposome Inc.) and analyzed using GCxGC/ToF-MS (Pegasus 4D from LECO) with target and nontarget techniques. Volunteers for sampling were recruited from local high school institutions located in three areas of the Santiago Metropolitan Region with different socioeconomic characteristics, according to the latest population census, and at more than 15 km from each other. Volunteers (5 from each high school selected) were students living within 3 km of the institution, that walked to school and back everyday, and that did not leave their neighborhood during the exposure week. Silicon wristbands were deployed during a full workweek (Monday to Friday) during summer and winter, and later extracted using a simplified method with ethyl acetate in an orbital shaker. Extracts were concentrated to 1 mL and analyzed directly in the GCxGC/ToF-MS without further processing. More tan 800 chromatographic features were detected in all silicon wristbands within a group (neighborhood), and included personal care products, polycyclic aromatics, pesticides, insecticides and others (according to their MS and NIST library hit). However, due to the lack of enough standards, only ~30 compounds were confirmed with authentic standards (retention times and MS). The resulting compounds were classified into groups based on their uses (according to the PubChem database online), and its distribution showed seasonal and spatial variations that could be attributed to lifestyle differences. The results will be used to create the first database of vapor phase organic compounds to describe a city or neighborhood ("fingerprint"). This effort will be replicated in other cities to compare the results.

**TP049 Artificial turf: Volatile and semi-volatile chemical movement and development of silicone wristband partitioning coefficients**

*C. Donald, R. Scott, G. Wilson, P. Hoffman, K.A. Anderson, Oregon State University / Environmental and Molecular Toxicology*

This work provides the first quantitative measure of in situ flux of semi-volatile contaminants on artificial turf fields. Passive samplers were used to identify gas-phase PAHs and OPAHs not previously reported associated with artificial turf. Utilizing a broad and targeted screen, we assess both artificial turf and from crumb rubber for 1,529 chemicals, including several with known health effects including benzo[c]fluorene. We also report the presence of 25 chemicals that have not yet been reported in artificial turf literature, including some with known effects on human health. This is the first report of bioavailable gas-phase PAH and OPAH concentrations on an outdoor field, to date gas-phase concentrations have only been reported from indoor facilities. Turf air and air were highly correlated at all three sites, and particularly at the recently-installed indoor site. Finally, thermal extraction and silicone passive samplers are highly suitable for larger-scale sampling campaigns that aim for less solvent and sample processing. We demonstrate for the first time that silicone passive samplers can be used to quantify volatile and semi-volatile organic chemicals from artificial turf. Co-deploying silicone passive samplers and conventional low density polyethylene, we develop partitioning coefficients that can be used for silicone passive air sampling environmental assessment.

**TP050 Acute Cardiovascular Effects of Smoke Inhalation in Firefighters**

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Firefighters comprise the largest group of public safety employees with more than 1,000,000 career and volunteer firefighters in the United States. Among this occupation, cancer and cardiopulmonary diseases account for more than half of work-related deaths, with smoke inhalation being the primary risk factor. Even though biomass smoke inhalation is shown to increase disease incidence, the comprehensive impacts of exposure and the components(s) responsible for disease outcomes are not well understood. During active fires, firefighters often work within the smoke plume to continuously manipulate the fire front. Respiratory protection is rarely practiced due to the weight and limited air capacity of most respirators. As a result of their proximity to active fires and lack of respiratory protection, wildland firefighters are exposed to hazardous pollutants such as aerosolized carcinogenic chemicals (e.g. polycyclic aromatic hydrocarbons (PAHs), formaldehyde, and benzene) as well as other irritants and toxins (acrolein, PM, and carbon monoxide). According to IARC, firefighting exposures are classified as possibly carcinogenic to humans (Group 2B). Smoke particles may trigger systemic inflammation and oxidative stress through formation of reactive oxygen species (ROS). ROS may induce endothelial dysfunction in arteries, leading to physiological function deterioration. The characterization of smoke exposures with biological (or clinical) monitoring is limited due to the inherent hazards of interfering with firefighting tasks in an active wildfire environment. This project utilized the strengths of in situ occupational exposure monitoring and health assessments to determine the effects of biomass smoke's PM on cardiovascular function impairment in wildland firefighters. We utilized continuous lightweight particle counting monitors to determine personal exposures to PM. Changes in cardiovascular function were measured continuously by heart monitors and ambulatory blood pressure monitors. During this study, firefighters were exposed to PM ranging from 0.03 to 10.3 mg/m$^3$ over periods of 4 to 7 hours. We found cross-shift increases in heart rate and decreases in blood pressure indicating acute cardiovascular effects after biomass smoke inhalation.

**TP051 Chemical characterization and potential risks from air emissions at hydraulic fracturing operations in Alberta**

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Hydraulic fracturing fluids have been successfully used for many years to increase well-site productivity in tight oil and gas formations. Once the reserve is sufficiently fractured to release the oil or gas from the geological formation the fracturing fluids must be returned or "flowback" to surface. At surface the liquid and gaseous components are separated, liquids are stored while gases and aerosols are combusted through flaring and released to the ambient environment. In Alberta, the annual average volume of gas flared to the environment from horizontal multistage fractured oil and gas wells is 167.6 million m$^3$. To date hydraulic fracturing (HF)technology has been used at more than 180,000 oil and gas wells in Alberta. As the intensity of hydraulically fractured oil and gas well development increased, so too did concerns of potential adverse health effects in nearby residents and livestock. The anxiety and anger over noted health effects in residents in rural Alberta has not ceased. Provincial government has also expressed concerns about the possible deterioration of air quality in proximity to HF wells and adverse health effects in exposed populations.The provincial regulator has published 2 separate reports of recurrent human health complaints to identify the cause of resident's concerns. Neither of these reports could address the risks from air emissions at HF operations as pre and post flare emission streams had not been characterized. The data gap forced the provincial regulators of health and the environment to initiate studies to characterize both the pre- flare stream and a partial post combustion stream. For the first time chemical characterization including inorganic (cations, anions, radon, metals) and organic (chlorinated and brominated additives, VOCs, PAHs) gases and aerosols in the pre and post flare streams at hydraulic fracturing operations in Alberta will be presented. The data will be presented in a risk-based context focusing on potential linkages to reported health effects of residents of Alberta.

**TP052 Molecular profiling of electronic cigarette liquids and vapors by NMR spectroscopy**

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Electronic cigarettes (e-cigs) have been designed to mimic conventional cigarette and deliver nicotine vapors (e-vapors) without the harmful tobacco byproducts such as tar and polycyclic aromatic hydrocarbons (PAHs). In addition to nicotine, e-cig liquids (e-liquid) contain propylene glycol, glycerin, water and unidentified chemicals used as flavors and preservatives. Some of them are classified as "generally regards as safe" chemicals as food additives; however, their effects when inhaled are unknown. Moreover, e-vapors may contain hazardous and carcinogenic byproducts formed during heating. The variability in e-liquids composition, conjointly with inconsistent user patterns and conditions, may trigger adverse exposure conditions to e-vapors of users and secondhand groups. The objective of the study is to determine the molecular composition of e-vapors and relate it to the e-liquid chemical fingerprinting and e-vapor generation conditions. To achieve this, a rapid non-destructive methodology has been developed for the spectral recognition of the major components (i.e. nicotine, propylene glycol and glycerin) as well as flavors and byproducts formed during e-vapors generation. The limit of detection, quantification, accuracy, precision, bias and uncertainty were determined using authenticated standards. E-vapors from 43 e-liquids were generated by a single port e-cigarette exposure system using the puff protocol (4 sec duration puffs every 30sec with a total puff volume of 55ml). Particle number and size distribution were monitored by continuous analyzers. E-vapors were collected of filter media and analyzed by 1D $^1$H and 2D $^1$H-$^1$H and $^1$H-$^{13}$C NMR spectroscopy. E-liquids spectra were dominated by propylene glycol and glycerin resonances; however, resonances in the aromatice and carbonyl regions were also observed. Menthol and vanillin were identified in most of the e-liquids. Elevated particle number concentrations were associated with e-vapors with most of them in the accumulation mode (with diameter from 0.5-1 µm). In addition to propylene glycol and glycerin, nicotine, benzene and toluene were also detected in the range of 100–38,000 ppbv/puff. The findings of this study demonstrate the potential for inhalation of large quantities of chemicals with unknown toxicological responses in the respiratory tract and the generation of known carcinogens, supporting the immediate need to better characterize the composition and toxicological profiles of e-vapors.

**TP053 Size distribution and clothing-air partitioning of polycyclic aromatic hydrocarbons generated by barbecue**

*J. Lao, Jinan University*

Barbecue (BBQ) is one of the most popular cooking activities with charcoal worldwide and may produce abundantpolycyclic aromatic hydrocarbons (PAHs) and particulate matter. Size distribution and clothing-air partitioning ofparticle-bound PAHs are significant for assessing potential health hazards to humans due to exposure to BBQ fumes, but have not been examined adequately. To address this issue, particle and gaseous samples were collected at 2-m and 10-m distances from a cluster of four BBQ stoves. Personal samplers and cotton clothes were carried by each participant sitting around the BBQ stoves. Particle-bound PAHs (especially 4−6 rings) derived from BBQ fumes were mostly affiliated with fine particles in the size range of 0.18−1.8 µm. High molecular-weight PAHs were mostly unimodal peakingin fine particles and consequently had small geometric mean diameters and standard deviations. Source diagnostics indicated that particle-bound PAHs in BBQ fumes were generated primarily by combustion of charcoal, fat content in food, and oil. The influences of BBQ fumes on the occurrence of particle-bound PAHs decreased with increasing distance from BBQ stoves, due to increased impacts of ambient sources, especially by petrogenic sources and to a lesser extent by wind speed and direction. The present study also demonstrated that Ant/(Ant+Phe) may be a good indicator of distance variability from emission sources. Octanol-air and clothing-air partition coefficients of PAHs obtained from personal air samples were significantlycorrelated to each other. High molecular-weight PAHs had higher area-normalized clothing-air partition coefficients in cotton clothes, i.e., cotton fabrics may be a significant reservoir of higher molecular-weight PAHs.

**TP054 Occupational paint exposure: Implication on blood total antioxidant capacity and antioxidant enzymes activity in Nigeria**

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Numerous harmful complex mixtures of chemicals and heavy metals; benzene, plasticizers, chromium, Cadmium and lead oxides have been reduced or substituted in paint in some countries, although they are still used in some developing countries like Nigeria. Evidence is rising continually concerning the adverse health effects of exposure to toxic chemicals in paint, yet it has not been adequately addressed in Nigeria. The aim of this study is to determine the effect of occupational exposure to paint on blood level of some antioxidant enzymes and Total Antioxidant Capacity (TAC), as an assessment of health risk. By random sampling, volunteers consisting of thirty (30) male painters aged between 20 to 50 years and 30 age-matched male controls were selected for the study. Five (5) ml of venous blood were collected from all participants for determination of serum Antioxidant enzymes; superoxide dismutase (SOD), catalase and glutathione peroxidase activities and also Total Antioxidant Capacity (TAC) using ELIZA techniques. SOD, catalase, Glutathione peroxidase and TAC levels in exposed group were significantly decreased($p=0.000$ in each case) in Cement Workers on comparison with control group. Comparison of the antioxidant enzymes in paint workers with < 5 years' exposure versus paint workers with ≥5 years' exposure shows no statistical difference in serum SOD, glutathione peroxidase, and TAC ($p=0.095$, $p=0.945$ and $p=0.837$ respectively). But there was significant increase in serum catalase level of paint workers with >5years exposure compared to those with < 5years exposure ($p=0.038$). There was no significant correlation of serum TAC with SOD, Catalase and Glutathione Peroxidase in Paint Workers ($r=0.059$, $P=0.784$; $r=0.077$, $P=0.720$ and $r=0.051$, $P=0.812$ respectively). It appears that occupational exposure to complex mixture of solvents and heavy metals contained in paint may lead to generation of free radicals, increase in oxidative stress among the paint workers, and thus may contribute to the observed decreases in antioxidant enzymes and TAC activities, thereby predisposing them to adverse health effects

**TP055 The Unexpected Way Alligators Can Harm you**

*E. Madonick, Brooks Applied Labs*

Industrial polluters play a major role in the effects of ecotoxicity translated to apex predators. Bioaccumulation of harmful metals up the food chain is an alarming human-health concern, particularly in at-risk populations such as young children and women of child-bearing age. Alligator meat has been touted as a healthy protein alternative to beef, chicken, and pork and is rapidly becoming more popular with gourmet options featured in restaurants and recipes online. Additionally, there is a large underrepresented population of subsistence hunters in the Southern United States that rely on alligator meat as the primary source of protein in their diets. In this study, alligator meat samples were collected from six different regions in Mississippi and Louisiana. Mercury, monomethylmercury, and additional elements of potential concern were evaluated with regards to physical and spatial attributes.

**TP056 Persistent and Toxic Chemical Pollutants in Fish Consumed by Asians in Chicago**

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Fish in contaminated waterways contain persistent and bioaccumulative toxicants such as mercury (Hg), polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polybrominated diphenyl ethers (PBDEs) and other halogenated flame retardants (XFRs). Ingesting these contaminants via fish consumption increases the risk of cancer, cardiovascular disease, prenatal developmental abnormalities, and other health problems. Compared with other racial/ethnic groups in the U.S., Asians consume seafood more frequently. However, there is little information about how culturally specific fish consumption contributes to exposure to these toxins. As part of a large-scale assessment and intervention project among the Asian population in Chicago, we obtained 103 seafood samples from local vendors (supermarkets, fish markets, Asian markets) and analyzed each sample for 66 PCBs, 17 OCPs (3 DDT related compounds, 4 HCHs, and 10 others), 8 PBDEs, and 9 other XFRs with the use of gas chromatography coupled with triple quadrupole mass spectrometry (GC/QQQMS). Total Hg was measured by a DMA-80 direct mercury analyzer. The samples covered 21 different types of fish and foodstuff including unusual fish species such as eel and fish cakes, and were selected based on a survey on fish consumption among Chinese, Korean and Vietnamese communities in Chicago. Among organic chemical groups, the rank order of concentration in all samples was $S_{66}$PCBs > $S_{17}$OCPs > $S_{8}$PBDEs > $S_{9}$XFRs. Spearman correlations among analyte groups were statistically significant at 95% confidence level, with the exceptions of those between $S_{4}$HCHs and $S_{66}$PCBs, $S_{8}$PBDEs, or $S_{9}$XFRs, and between Hg and $S_{17}$OCPs. Among the individual organic analytes, DDT was the highest in both median (0.7 ng/g wet weight) and mean (2.1 ng/g ww) concentrations, and cis-permethrin, a currently available insecticide, had the highest detection frequency (95%). The rank of the sample types varied by chemical groups. Fish with highest mean Hg (> 1 mg/kg) were bluefish and pike. The mean $S_{66}$PCBs was highest in pike and bluefish (>100 ng/g ww) followed by pollock and mackerel (>40 ng/g ww); these fish species were also more contaminated by flame retardants than others. The highest $S_{17}$OCPs (>40 ng/g ww) were observed in pompano and yellow croaker. Overall, octopus, shrimp, and tilapia were the least contaminated. Results of this analysis will inform future exposure assessments and the development of intervention strategies.

**TP057 Mixed-Organic/Inorganic-Chemical Exposure in USA Point-of-Use Drinking Water**

*P.M. Bradley, USGS / South Atlantic Water Science Center; D.W. Kolpin, USGS / Central Midwest Water Science Center; K. Romanok, USGS; K.L. Smalling, USGS / NJ Water Science Center*

Drinking-water from 25 sites (13 home, 12 workplace) in 11 US states was assessed at the point of use (tapwater) for 482 organics and 19 inorganics, with 6 home samples from untreated, self-supply. Seventy-one organics were detected in at least one tapwater sample, some exceeding drinking water Maximum Contaminant Level Goals. Two inorganics were frequently detected at concentrations exceeding drinking water Maximum Contaminant Level Goals. A multiple lines of evidence approach was used to evaluate potential human-health concerns of detected tapwater chemicals. These results document the widespread potential in the US for human exposure to a variety of previously uncharacterized contaminant mixtures in tapwater.

**TP058 Identifying agricultural pesticides from tap water and household dust samples in Knights Landing utilizing GC-qTOF-MS and LC-qTOF-MS**

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Pesticide usage in Knights Landing, CA is a community health concern based on close proximity between residences and surrounding agriculture, particularly due to drift from aerial spraying.Based on Pesticides Information Portal data, an annual average of 997,540 pounds of pesticideactive ingredients were sprayed between 2011 and 2015 in the zip codes surrounding Knights Landing. The aim of this project is to better understand the significance of aerial spraying withexposure in nearby communities. Contamination of well-sourced tap water and accumulation ofpesticides in indoor dust are theorized to be major sources of household sources of exposure.Detection of pesticides in tap water from private wells would suggest leaching to the groundwater, while detection in household surface dust would suggest wind travel or occupant delivery. Current monitoring of pesticides in tap water or household dust is limited to the small public water system in Knights Landing. Using both targeted and suspect GC-qTOF-MS and LC-qTOF-MS analysis we are able to perform a holistic chemical analysis of these samples. The target analysis will consist of fourteen pesticides including Diuron, Bifenthrin, and Chlorothalonil. We will also perform suspect analysis by screening the data through a personal compound database library (PCDL) containing spectral information for over 1,600 pesticides and their degradation products. Five tap water and five household dust samples were collected fromKnights Landing homes for this study. This poster outlines the methods and workflow used for water and dust extractions, as well as for both targeted and suspect data analysis. This study addsto our understanding of the residential exposure to pesticides in intensive agricultural areas of California. Supported by P30 ES023513 and T32 HL007013

**TP059 Characterizing Azobenzene Disperse Dyes in Indoor Environments**

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Disperse dyes are a class of substituted anthroquinone- or azobenzene-based dyes used to color synthetic fabrics such as polyester, nylon, and acrylic. Many of the dyes are chlorinated or brominated and account for roughly 70% of the 9.9 million tons of industrial dye colorants used annually. Azobenzene dyes are well-characterized as electrophilic mutagens and contact allergens in clothing and in the aquatic environment, but little is known about occurrences and health implications of these dyes in the indoor environment. Here, we report on the concentrations of several azobenzene dyes in house dust samples collected from 190 homes in the Toddlers Exposure to SVOCs in Indoor Environments (TESIE) study in central North Carolina (2014-2016). Five representative azobenzene dyes and two transformation products were quantified in the TESIE dust samples to assess children's exposure to dyes in the indoor environment. House dust samples were collected for each study participant's household using standardized protocols. All samples were analyzed for Disperse Blue 373, Disperse Orange 25, Disperse Orange 37, Disperse Orange 61, and Disperse Violet 93, and for the transformation products (TPs) 2,6-dibromo-4-nitroaniline and 2-bromo-4,6-dinitroaniline. Dyes were analyzed via HPLC-ESI-HRMS/MS and quantified via HPLC-APCI-MS/MS. One or more dyes were detected in 92% of house dust samples. Quantitative measurements revealed that these dyes and TPs are present at levels up to 10 µg/g in house dust. Geometric means ranged from 50 ng/g to 254 ng/g house dust, with Disperse Blue 60 having the highest concentration overall. In addition, several brominated dyes were found at levels known to exhibit mutagenicity and concentrations were similar to ranges reported for brominated flame retardants. These results suggest that children's exposures to disperse dyes in house dust is common, and warrants further detailed study and characterization. Future work in our laboratories will examine the potential for disperse dyes to act as respiratory allergens at environmentally relevant concentrations.

**TP060 Azoxystrobin Exposure in the Indoor Environment: Is Drywall a Source?**

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Azoxystrobin is a fungicide that belongs to the class of chemicals known as strobilurins, which were originally derived from the fungus *Strobilurus tenacellus*. Today the strobilurin pesticides are a high production volume fungicide used primarily in fruits and vegetables. However, a recent study raised concerns about the potential for these chemicals to cause neurotoxicity. Interestingly, azoxystrobin now has a patent for use in wallboards (i.e. drywall) to prevent mold growth, but it's unclear whether or not these fungicides would migrate into indoor environments. The purpose of this study was to determine if azoxystrobin could be detected in new drywall samples, and whether the strobilurins would be detected in samples of house dust. To support this study we collected 10 dry wall samples intentionally marketed for use in bathrooms to inhibit mold, and analyzed 191 house dust samples collected through a children's health study in central North Carolina in 2014-2016. The drywall and house dust samples were extracted and analyzed for four strobilurin pesticides, including azoxystrobin, pyraclostrobin, trifloxystrobin and fluoxastrobin using liquid chromatography tandem mass spectrometry. Detection frequencies in house dust for the four fungicides ranged from 36 – 93%. Azoxystrobin was detected most frequently and at the highest concentrations with a geometric mean of 3.4 ng/g and a maximum concentration of 10,590 ng/g. The geometric means and ranges for pyraclostrobin, fluoxastrobin and trifloxystrobin were 0.51 (< MDL – 35.6), 0.21 (< MDL- 40.7) and 0.05 (< MDL -2.6 ) ng/g, respectively. Azoxystrobin was also detected in newly purchased purple wallboard samples at concentrations near 1% by weight. Ongoing analyses are examining the variability in dust concentrations for azoxystrobin based on the age of the home, and the presence of this type of wallboard in the home. These results suggest that fungicides present in wallboard may be migrating to the indoor environment, leading to exposure in the residences that would constitute a separate exposure pathway independent of dietary exposures.

**TP061 Human Exposure to Phthalate Esters (PAEs) in Social Housing Apartments**

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Phthalate esters, widely used as plasticizers and additives in building materials and consumer products, have been found to cause a range of adverse health effects at ambient levels. Many indoor sampling studies have focused on homes from mid-to-high-socio-economic status (SES) occupants. Residents of low-income social housing are vulnerable to health effects from exposure to indoor air pollution and yet have received much less attention. This study documented concentrations of eight phthalates (DEP, DiBP, DnBP, BzBP, DEHP, DnOP, DiNP and DiDP) in indoor air with the larger goal of assessing indoor exposure in lower income social housing. Silicone rubber (polydimethylsiloxane or PDMS) passive air samplers were deployed for one week in 71 units in 7 multi-unit social housing buildings located in Toronto, Canada, during the late spring of 2017. Three types of units were sampled: bachelor, senior and family apartments. Sixty-five of the 71 PDMS samplers deployed were recovered for analysis on a GC-MS in EI mode. Chemical mass collected by the samplers was converted to air concentrations. All eight phthalates were detected in all the PDMS samples from 65 units. DEP (found in fragranced products) was the dominate phthalate in indoor air with a geometric mean concentration of 2,160 ng/m$^3$, followed by DnOP (1,440 ng/m$^3$) and DiBP (1,200 ng/m$^3$), which are mainly used as plasticizers. Concentrations of DnBP, DnOP and DiNP in senior and family units were significantly ($p < 0.05$) higher than those in bachelor units. In general, residents in social housing units experienced two to 16 times higher air concentrations of phthalates than those of predominantly detached and semi-detached houses in Toronto (presumably occupied by occupants with higher SES and with lower occupant densities).

**TP062 Estimating human exposure to di(2-ethylhexyl)phthalate in indoor environment using an indoor exposure model at different temperature**

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Although di(2-ethylhexyl)phthalate (DEHP) has adverse health effects on humans, it is still widely used as a plasticizer in many polyvinyl chloride (PVC) products. Because DEHP is a semi-volatile organic chemical (SVOC), it is emitted from PVC flooring in indoor environment. Human may be exposed to DEHP via direct inhalation of gaseous DEHP or ingestion of indoor dust particles sorbing DEHP. To estimate the exposure to SVOCs, an indoor exposure model is used. To predict the concentration of SVOCs in indoor environment using a model, the emission rate is an important parameter and is related to the diffusion coefficient of SVOCs in PVC flooring material. The emission rate may change with temperature because the diffusion coefficient is temperature-dependent. Even though the room temperature does not change much, PVC floors are heated directly to warm home in Korea during winter season. To apply this home-heating conditions in Korea, the concentrations of DEHP in indoor air were estimated using an indoor exposure model at various contents and the diffusion coefficients of DEHP in PVC flooring as parameters to estimate emission rate at different temperature. The exposure model was developed using mass balance equations which account for the accumulation of DEHP in indoor environment. The migration rates of DEHP from PVC flooring to a passive sampler using polydimethylsiloxane at different temperature (20, 25, 40, and 60 °C) were measured to obtain the diffusion coefficients of DEHP in PVC flooring materials. The indoor air concentrations of DEHP at steady state were estimated 0.05, 0.25, and 0.49 µg m$^{-3}$, and the concentrations sorbed to indoor dust were 0.014, 0.069, and 0.137 $\mu g_{DEHP}\ \mu g_{dust}^{-1}$ when the contents of DEHP in PVC flooring are 1, 5, and 10 % respectively at 25 °C. The inhalation exposure and dust ingestion exposure to DEHP were estimated 0.22 and 1.22 µg kg$^{-1}$ day$^{-1}$ for one-year-old baby whose body weight is 11 kg and inhalation rate is 9.75 m$^3$ day$^{-1}$. The diffusion coefficients of DEHP in PVC flooring were measured as $2.76 \times 10^{-17}$, $6.14 \times 10^{-17}$, $4.95 \times 10^{-16}$, and $2.90 \times 10^{-15}$ cm$^2$ sec$^{-1}$ at 20, 25, 40, and 60 °C respectively. Stiff increase in diffusion coefficient in PVC flooring with temperature suggests that the emission rate of DEHP from PVC flooring would dramatically increase under the home-heating condition during the winter, leading to elevated intake rates via gas inhalation and dust ingestion.

**TP064 Triphenyl phosphate promote the development of atherosclerosis and foam cell formation**

*W. Hu, H. Ma, J. Hu, Peking University*

Triphenyl phosphate (TPHP), one of the organophosphate flame retardants (OPFRs), has been frequently detected in the environmental media and even in the blood of the general population. It has been reported that TPHP could cause various adverse effects such as endocrine disruption and obesity. In our study, we evaluated plasma lipid change and the severity of atherosclerosis based on the morphological and histological changes that occurred in a well establised mode of atherosclerosis (ApoE-/- mice). We treated the mice with 10 mg/kg bw and 40 mg/kg bw TPHP for 13 weeks. Results showed that 40 mg\kg bw TPHP exposure can significantly increase the level of total cholesterol and triglycerides in serum. TPHP can significantly promote the development of atherosclerosis in mice at the dose of 10 mg\kg bw and 40 mg\kg bw. Since foam cell formation plays a central role in the development of atherosclerosis, we determined the foam cell formation induced by TPHP, and found that TPHP can significantly promote the formation of foam cell in vitro. Our study show that TPHP can promote foam cell formation and atherosclerosis, and call for further study of the toxicological effects of TPHP on human health.

**TP065 Asthma risk associated with the US 2012 heatwave: A local health department prespective**

*L. Figgs, Douglas County Health Department / Environmental Health*

*Background*: Public concern about global climate change is forcing local public health agencies to assess how climate change might impact local disease risk.*Objective:* Estimate local asthma diagnosis risk associated with the 2012 heatwave.*Methods:* This is a retrospective, observational, epidemiologic investigation using a case-control study design. Subjects are selected from Douglas County NE hospital emergency department (ED) admissions in 2011 and 2012. Risk is estimated by conditional logistic regression. *Results*: Overall, asthma ED diagnosis odds were 1.23 (95% CI; 0.96-1.57) times higher in 2012 compared to the same 2011 calendar period. Asthma ED diagnosis odds were 3.37 (95%CI=2.27-4-17) times higher among subjects < 19 years old compared to subjects ≥ 19 years old, and 3.25 (95%CI=2.63-4.02) times higher among African-Americans than non-African-Americans. Absolute humidity was inversely related to asthma diagnosis risk ( 2 = 16.6; p< 0.001). *Conclusion:* Asthma diagnosis risk was elevated in Douglas County's 2012 ED.

**TP066 Effect of aqueous extract of cocos nucifera oil on sex hormones and some markers of oxidative stress in drug-induced testicular toxicity in rats**

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Human spermatozoa, as recorded, appears to suffer from oxidative stress with impact on the normality of their functions and the integrity of their nuclear and mitochondria DNA. Objective: This study was aimed at investigating the effect of Virgin coconut oil (VCO)on ciprofloxacin-induced testicular toxicity and oxidative stress. Method: 36 male Albino rats (weighing between150-200g) were divided randomly into four experimental groups ; group I, received 1ml of distilled water. Group 2 received 130 mg/kg B.W of ciprofloxacin. Group 3 received 130 mg/kg B.W of ciprofloxacin and 10 ml/kg B.W body weight of virgin coconut oil, 30 minutes after ciprofloxacin, while group 4 received 10ml /kg B.W of virgin coconut oil only. All animals were allowed access to normal food and water ad libitum. The administration was done orally for a period of 25days. Semen samples were collected at the end of the experiment and used for the analysis of sperm count and sperm motility. Blood samples were also collected for the determination of testosterone, FSH ,LH, superoxide dismutase activity and serum malondialdehyde by direct spectrophotometric method(for SOD and MDA) and ELISA assay( for Testosterone, FSH and LH ). Result: The results obtained shows that there were statistically significant decrease in sperm concentration , motility, serum testosterone and superoxide dismutase activity in group 2 when compared to the groups 1 and 4. Significant increase was observed in the mean levels of malondialdehyde, FSH and LH in group 2 when compared to those of groups 1 and 4 at P< 0.05. The mean levels of sperm concentration, motility, ,testosterone , and superoxide dismutase were significantly increased in group 3 when compared to those of group 2 . The serum levels of malondialdehyde, FSH and LH decreased significantly in group 3 when compared to group 2. Conclusion: Results demonstrated that Ciprofloxacin induced poor sperm characteristics, in addition to reproductive hormone imbalance in male rats. However this stress induced toxicity was eventually ameliorated by Virgin coconut oil . VCO can therefore be recommended as supplement in the management of ofloxacin induced hormonal imbalance and oxidative stress in males.

**TP067 Simultaneous analysis of seven biomarkers of oxidative damage to lipids, proteins, and DNA in urine**

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The determination of oxidative stress biomarkers (OSBs) is useful for the assessment of health status, exposure to emerging contaminants and progress of diseases in humans. Whereas previous methods for the determination of OSBs in urine were focused on a single marker, in this study, we present a method for the simultaneous determination of biomarkers of oxidative damage to lipids, proteins, and DNA. 2,4-Dinitrophenylhydrazine (DNPH) derivatization followed by solid phase extraction (SPE) and high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) allowed the determination of 8-hydroxy-2'-deoxyguanosine (8-OHdG), o-o'-dityrosine (diY), malondialdehyde (MDA), and four $F_2$-isoprostane isomers: 8-iso-prostaglandin$F_{2\alpha}$ (8-$PGF_{2\alpha}$), 11β-prostaglandin$F_{2\alpha}$ (11-$PGF_{2\alpha}$), 15(R)-prostaglandin$F_{2\alpha}$ (15-$PGF_{2\alpha}$), and 8-iso,15(R)-prostaglandin$F_{2\alpha}$ (8,15-$PGF_{2\alpha}$) in urine. Derivatization with DNPH and SPE were optimized to yield greater sensitivity and selectivity for the analysis of target chemicals. The limits of detection of target analytes in urine were below 30 pg $ml^{-1}$. The assay intra- and inter-day variability was below 16% of the relative standard deviation, and the recoveries of target chemicals spiked into synthetic urine were near 100%. The method was applied to the analysis of 21 real urine samples, and the analytes were found at a detection frequency of 85% for 8-$PGF_{2\alpha}$ and 15-$PGF_{2\alpha}$, 71% for 11-$PGF_{2\alpha}$, 81% for 8,15- $PGF_{2\alpha}$ and 100% for diY, 8-OHdG, and MDA. This method offers simultaneous determination of multiple OSBs of different molecular origin in urine samples selectively with high accuracy and precision.

## Environmental Stewardship and Economic Development: How We Can Accomplish Both

**TP068 Buffalo River Remediation, Restoration and Revitalization**

*R. Davis, Anchor QEA LLC / Natural Resources; R. Mohan, M. Reemts, Anchor QEA LLC*

Over 500,000 cubic yards of contaminated sediments were removed from the Buffalo River AOC as part of a Great Lake Legacy Act project. Remediation was performed as a collaboration of GLNPO, Honeywell, and USACE and involved dredging and capping adjacent to riverfront structures and shorelines and coordinating work with property owners, the Buffalo Niagara Waterkeeper and the City of Buffalo. The project also included a significant habitat restoration component that was designed to augment ongoing and planned restoration projects at adjacent sites. The combination of remediation and restoration has increased public use of the shoreline areas and waterways which are part of the Buffalo "Blueway." The Blueway is in integral part of the Buffalo Revitalization Strategic Plan to connect citizens with the shoreline and river and generate interest and investment for revitalizing riparian properties.

**TP070 Prioritizing Site Remediation and Restoration to Achieve Economic and Conservation Goals**

*S.S. Brown, The Dow Chemical Company / Environmental Remediation and Restoration*

Corporate environmental remediation managers are charged with investigating and addressing contaminant risks in order to achieve effective site remediation and liability closure quickly and cost effectively. Corporate and regulatory managers might emphasize differing priorities, but all would do well to focus early on options and opportunities for future uses for these properties. Sites in urban areas frequently provide opportunity for economic redevelopment, subject to any necessary restrictions. Redevelopment options are often prioritized in urban settings, but these sites also afford the chance to restore and protect riparian buffers and floodplains (green infrastructure) and to reconnect neighborhoods with natural resources. Redevelopment and environmental stewardship are not mutually exclusive! In cases where large corporate land holdings are located in more remote and ecologically sensitive areas, which may or may not be subject to remediation, these lands sometimes have the potential to support high-priority, regionally significant conservation objectives. Within the Environmental Remediation and Restoration Group at Dow, we have prioritized planning for future land use that provides a mix of redevelopment and green infrastructure at urban sites, and maximizes conservation value and ecological services at more remote sites. In this presentation, ongoing projects will illustrate a collaborative approach that utilizes scientific research with academic partners to address site-specific risks, and interactions with land planners and conservation partners to create future use options that maximize economic and ecological value, as appropriate for each site. Trust derived from these partnerships and mutually beneficial future use scenarios help create a project climate that can serve to expedite site remediation/restoration, closure, and subsequent transaction for redevelopment and/or conservation

**TP071 Enhancing Ecosystem Services in the Hackensack Meadowlands through Remediation and Restoration**

*R. Davis, Anchor QEA LLC / Natural Resources; S.S. Brown, The Dow Chemical Company / Environmental Remediation and Restoration*

The Hackensack Meadowlands is one of the most recognizable wetland complexes in the United States. While drastically reduced in size since the 1700s due to multiple anthropogenic impacts, the Meadowlands provides valuable ecosystem services to the region. These services include flood control and storm protection, sediment and nutrient retention and export, and ecological habitat including the Atlantic Flyway for migratory birds. Restoration of the wetland and ecosystem functions in this heavily urbanized watershed has been an agency and community goal for decades, and there are several examples of restored wetland areas throughout the Meadowlands. Restoration efforts have had to overcome challenges such as Phragmites, dams, ditched and diked tributaries, contaminated sediments, and most recently changes associated with sea level rise and major storms including Superstorm Sandy. The Meadowlands are also home to several active contaminated sediment sites. Remedial investigations and feasibility studies of these sites are in progress or planned. Management of these sites provides an opportunity to evaluate and plan remediation in the context of the larger watershed-scale initiatives to reduce risk, restore ecosystem functions, and provide resiliency to counter sea level rise and climate change. This presentation will provide examples from lacustrine, riverine and estuarine systems where restoration has been integrated with sediment remediation to reduce risks to ecological and human health and enhance ecosystem services. Examples on how this approach can be integrated with other coastal resiliency initiatives (e.g., Rebuild by Design) will also be discussed.

**TP072 Novel Analysis of Pollution Prevention Data in New Jersey**

*D.R. Millemann, R. Mueller, N. Procopio, New Jersey Department of Environmental Protection / Division of Science, Research & Environmental Health*

Pollution prevention (P2) planning is a regulatory tool used to promote economic benefits for manufacturing facilities, as well as environmental benefits for the public. Facilities are required to report chemical data annually to satisfy regulatory requirements, and researchers and policy makers use these data to assess the success of the regulation and design future regulatory programs. P2 methodologies are well established; however, analysis of P2 data can be inconsistent and difficult to interpret due to the variability in reporting requirements from year to year. The New Jersey Department of Environmental Protection recently reviewed its P2 data to establish trends in the reduction of toxic compounds within the state. Facility data were analyzed for 1,009 facilities which includes 5,601 chemical processes. Non-product output (NPO), which includes on-site releases and off-site material transfers, was reported at 498 million pounds in 1996 and was reduced by 71.9% to 140 million pounds in 2009. The use (USE; total material consumed, shipped, and NPO) of reported chemicals decreased by 54.4% from a state maximum of 31.6 billion pounds in 1999 to 14.4 billion pounds in 2015. These values are subject to several changes in reporting requirements over time, and thus may not be representative of true losses (and/or gains) over the evaluation period. A novel approach was developed to determine reductions at a process level by evaluating the longevity and reductions during the life-cycle of processes. This approach more directly demonstrates the benefits of P2 planning while eliminating the variability introduced by inconsistencies in reporting requirements. During the first 10 years of P2 planning, USE decreased by 21.7% from the maximum in Year 2 of 25.9 billion pounds to 20.3 billion pounds in Year 10 for processes that existed for at least 10 years. For this same cohort, NPO decreased by 59.7% from 485 million pounds to 195 million pounds during 10 years of planning. Since its inception in 1991, New Jersey's P2 program has made great strides in facilitating the reduction of toxics use in the state. When analyzing P2 data, alternative methodologies could be implemented to ascertain meaningful trends. Establishing useful analyses and interpretations of data are fundamental to successful regulatory programs.

## Fate and Effects of Metals: Biogeochemical Perspective

**TP073 Metal toxicity during short-term sediment resuspension and redeposition in a tropical reservoir**

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Billings Complex is the largest water-storage reservoir in São Paulo, Brazil and has been contaminated from sewage and industrial effluents since the 1960s. Periodically, Billings sediments are subjected to currents causing resuspension which release metals. A short-term (4 h) resuspension was simulated using Sediment Flux Exposure Chambers (SeFEC) to better understand fate, bioavailability and transport of Fe, Mn, and Zn during these events and possible organism toxicity. Comparison studies consisted of contaminated and reference site sediments. *Daphnia magna* and *Hyalella azteca* were exposed during the 4 h resuspension, after 20 h redeposition, and monitored post-exposure for survival, growth and reproduction. Resuspension rapidly deoxygenated overlying water with decreasing pH, and resulted in an elevated dissolved Zn in the overlying water that remained above the USEPA criteria for acute toxicity (120 µg $L^{-1}$). However, Zn was almost totally scavenged (after 20 h) from solution as new sorption sites (i.e. OC or freshly-precipitated Fe oxyhydroxides) formed. Dissolved Mn increased continuously during and after resuspension with maximum values 20 h post exposure. An initial release of Fe occurred, likely associated with oxidation reactions of acid volatile sulfides, but decreased after 1 h of resuspension. The Fe decrease is likely due to precipitation as oxyhydroxides. As resuspended particles formed a freshly redeposited sediment layer, further oxidation occurred (up to 20 h), releasing Fe to the dissolved phase. No acute toxicity was observed during short-term resuspension; however, mortality was observed in *D. magna* and *H. azteca* 20 h post-exposure. *Daphnia magna* also showed chronic toxicity with decreased neonate production after post-exposure at both sites. This sub-lethal effect could lead decreased populations over a longer period. The adverse effects of resuspended contaminated sediments in hydrologically active systems, such as Billings reservoir, should be considered during environmental management decision-making.

**TP074 An Alternative to the Biotic Ligand Model to Explain Physiological Response to Binary Metal Mixtures in *Daphnia magna***

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We previously observed a non-additive protective interaction between cadmium and zinc in mixture studies in *Daphnia magna*. The observed acute toxicity of cadmium and zinc mixtures could not be explained by the individual toxicity of cadmium or zinc alone. We explored if the non-additive (protective) effect could be explained by competition between the metals consistent with a common biotic ligand model. We performed RNA-seq on daphnia exposed to each metal alone and a Cd/Zn mixture at the observed protective interaction doses. The gene expression of daphnia exposed to the Cd/Zn mixture was compared to the gene expression of daphnia exposed to cadmium only and zinc only. Our analysis of the RNA-seq data indicated that simple competition between Cd and Zn in a biotic ligand model may not fully explain the physiological (non-additive) effect. Rather, our data suggest that an organismal biologic response is the primary determinant of mixture effects. Daphnia exposed to moderately toxic doses of cadmium alone differentially expressed over 1000 genes (DEG's) at FDR < 0.1. Interestingly, we observed that in the Cd/Zn mixture, the number of DEG's was reduced to 291 DEG's as compared to Cd alone. Of these, 282 were shared in common between Cd and Cd/Zn mixture. Exposure to Zn alone at the same dose exhibited 24 DEG's. We suggest that these finding are not consistent with competition at a biotic ligand model. Biological pathway and cellular process analysis supports an organismal physiological response as playing a key role in the non-additive mixture. Diverse cellular responses were identified in the cadmium alone exposure that are consistent with the observed toxicity including induction of the unfolded protein response (UPR), antioxidant responses such as induction of glutathione biosynthesis, glutamate mediated signaling, and down regulation of genes related to digestive proteolytic enzymes. Importantly, several key responses appear to be mitigated in the mixture treatment group. Specifically, the induction of the unfolded protein response and the glutamate mediated signaling is not observed in Cd/Zn mixture. Some biological effects, such as the induction of glutathione are present in both the cadmium alone treatment and the mixture treatment group. These finding are consistent with a model in which cadmium is still bioavailable in the mixture but that Zn can prevent deleterious physiological responses. Overall, this work indicates that a biotic ligand competition model alone is unlikely to explain the explain non-additive interaction between cadmium and zinc and supports an important role for an internal physiological interaction between cadmium and zinc.

**TP075 Major physical and chemical drivers causing the spatial distribution of sediment metals in a large wave-dominated estuary in south-eastern Australia**

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Lake Macquarie, on the NSW central coast of eastern Australia is a large wave dominated estuary that has two distinct northern and southern regions that receive metal and metalloid contaminant inputs from a range of urban and industrial sources located within their respective catchments. The northern region of the lake has an extensive history of large scale industrial activity with a lead–zinc smelter, a fertiliser plant, a steel foundry, collieries and sewage treatment works having operated along and discharged to the northern lake's main tributary of Cockle Creek as well as receiving contaminants from marinas, stormwater inputs from urban and light industrial precincts and sewer overflows. In the southern region, significant inputs of contaminants (i.e. selenium, copper, zinc) come from ash dam overflows and cooling water releases associated with three large coal-burning Power Stations as well as inputs from urban and light industrial stormwater sources. In this paper, we describe the spatial distribution of sediment metals in both the northern and southern regions in relation to major point and diffuse sources as well as the physical and chemical drivers influencing mobilisation, dispersion and deposition. A better understanding of the factors driving observed distributions of metals in this estuary is needed to manage and mitigate the impact of anthropogenic borne contaminants on sediment quality.

## Coupling Models With Monitoring Data and Future Perspectives for Exposure Assessment

**TP076 Estimating Human Exposure to Pesticides in Drinking Water Using Spatial Groundwater Monitoring and Pesticide Use Reporting Data, or Groundwater Modeling**

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The Exposure Assessment Section (EAS) of the Human Health Assessment (HHA) Branch at the California Department of Pesticide Regulation (DPR) has developed a novel method for estimating the likelihood of human exposure to pesticides in California groundwater used as drinking water. Because groundwater exposure constitutes just a portion of aggregate exposure (which includes all potential exposures to a given pesticide due to ingestion, inhalation, and dermal contact), HHA may require more precise exposure estimates than those resulting from screening-level assessments (e.g., those used for pesticide registration reviews). Currently, HHA uses data from either the U.S. Department of Agriculture Pesticide Data Program or from the DPR groundwater Well Inventory Database to assess exposure, which may underestimate or overestimate levels of pesticide contamination in drinking water. The new method developed by EAS intends to increase the accuracy of groundwater exposure estimates. EAS proposes to derive the estimates from spatial groundwater monitoring and pesticide use reporting data, or from groundwater-solute transport modeling outputs. GIS analysis could be used for the former method to determine whether groundwater well sampling locations overlap with regions in which a given pesticide is used most heavily and whether the data are sufficient for estimating exposures. When monitoring data are lacking, exposures could be estimated based on computer simulation outputs, according to the pesticide's physicochemical properties, usage information, and site conditions (e.g., local weather; soil characteristics; etc.). EAS proposes to use a model of medium complexity, the Leaching Estimation And Chemistry Pesticide (LEACHP) model (Hutson 2003; Wagenet and Hutson 1989), because of its accessibility, precision (relative to screening-level models), and the availability of a California-specific set of environmental input parameters (developed by DPR). If some monitoring data are available, LEACHP outputs could be ground-truthed. EAS has also drafted a decision-making framework for determining whether monitoring data or modeling outputs will work best for a given pesticide and how to translate estimated pesticide residue concentrations into groundwater exposure estimates. Finally, a case-study assessment of the potential for human exposure to propyzamide (i.e., pronamide) by groundwater ingestion is included as a proof of concept.

**TP077 A Kinetic Mass Balance Model for Predicting Gas-Particle Partitioning of Low Volatility Organic Chemicals**

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The extent of long range transport of chemical pollutants in the atmosphere depends on their distribution between the gas phase and aerosols. Equilibrium partitioning between the gas and particle phases is assumed in most widely used multimedia chemical fate models. Assuming equilibrium has recently been shown to overestimate the fraction of very low volatility chemicals in the particle phase. Here, we present a regional multimedia chemical fate model that includes separate compartments for fine and coarse aerosols and the gas phase. We apply the model to investigate deviations from equilibrium partitioning for low-volatility polybrominated diphenyl ethers (PBDEs) by exploring model results for; 1) seven generic aerosol scenarios, and 2) emissions to the aerosol and gas compartments. When possible, we evaluate the model scenarios against field measurements of the distribution of PBDEs between the gas phase and aerosols. The distribution of low volatility chemicals deviated from equilibrium partitioning in each aerosol scenario. The extent of the deviation is determined by the distribution of emissions to the gas phase or aerosol phase and the deposition rate, diameter and organic carbon fraction of aerosols. The deviation increased with increasing fraction of emissions to the gas phase. In a scenario with 100% of emissions to the aerosol phase, the deviations are within a factor of 1.5, while the deviations increase to up to 10^6 when 100% of chemical is emitted to gas phase. By fitting the modeled chemical distribution between the gas phase and aerosols to measurements, our model predicts that low volatility PBDEs are emitted in the aerosol phase near an e-waste recycling facility in China and to the gas phase near urban areas in Italy and Turkey. The kinetic gas/particle partitioning model presented here can predict the distribution of low volatility organic chemicals between the gas phase and aerosols more accurately than equilibrium models, and it can be readily implemented in multimedia fate and transport models.

## Wildlife Forensics Toxicology

### TP078 Secondary poisoning by pentobarbital and flunixin in a vulture feeding station in Southeastern Spain

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Spain has the biggest colony of Griffon vultures in all Europe. The main cause of the decline in the griffon vulture population is the consumption of poisoned baits set out by people. However, sometimes another type of drugs can sometimes increase the number of individuals poisoned. Food supplied to in feeding stations is considered as a regular practice to maintenan of scavenger bird populations in those areas where food is scarce. On November 2016, the staff of the Wildlife Recovery Center “Santa Faz” found, in Alcoy feeding station (SE Spain), vultures with symptoms of weakness two days after supplying two carcasses of domestic goats. A total of five griffon vultures (Gyps fulvus) were submitted to the “Santa Faz” Wildlife Recovery Center (Alicante) to be medicated. All of them had a similar symptomatology: unconsciousness, hypothermia (between 37.7 ºC and 39.8 ºC), and shallow breathing. The treatment consisted of intravenous and subcutaneous administration of fluid therapy and maintenance of a warm environment for 3 days. Oral feeding was subsequently initiated on the basis of a soft diet with highly digestible protein. After 10 days under acute care they were released, completely recovered. Five days after the first registration, another vulture with strong symptoms of weakness was found in the feeding station. Fluid therapy was also applied in this vulture; however, it died 12 hours after admission in the WRC. At necropsy, whitish deposits were observed in the kidney, air sacs, liver and heart (visceral gout). Under suspicion of possible poisoning with a drug in the goats provided as food, the blood samples were analyzed. Blood samples of the five vultures were positive for sodium pentobarbital. After necropsy, the liver of the last one vulture was positive to flunixin, a non-steroidal antiinflammatory drug. Acknowledgements: Séneca Fundation MASCA’2014 Project (19481/PI/14). To Alvar Segui (father and son) for their invaluable service in the Canyet Project.

## Pesticide Runoff Pollution: Challenges and Opportunities in Prediction, Prevention and Management

### TP079 Effectiveness of an Integrated Wetland Treatment System in Reducing Pesticide Concentrations Associated with Agricultural Runoff

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The central coast of Monterey County, California, is a highly productive agricultural region within the Salinas Valley that produces much of the USA’s salad greens, artichokes, and cruciferous vegetables such as broccoli, cauliflower, and Brussels sprouts. Agricultural irrigation runoff containing pesticides found at concentrations toxic to aquatic organisms poses a threat to aquatic ecosystems within the local watersheds. This study was designed to monitor the effectiveness of a constructed wetland treatment system, which is additionally integrated with the floating aquatic plant pennywort (*Hydrocotyle spp.*) and granulated activated carbon (GAC) filtration, at the reduction of pesticides associated with agricultural runoff. The wetland system is supplied with water pumped from the Tembladero Slough, an agricultural drainage that eventually enters the Monterey Bay National Marine Sanctuary. Water samples collected throughout the wetland treatment system are analyzed for toxicity to the daphnid *Ceriodaphnia dubia*, the amphipod *Hyalella azteca*, and the midge *Chironomus dilutus*. Additionally, samples are analyzed for approximately 160 fungicides, herbicides and insecticides. Preliminary results show significant input toxicity to *H. azteca* and *C. dubia*, although daphnid toxicity was likely partially caused by elevated conductivity from tidal influence. Moderate reductions in chemical concentrations were observed with water-soluble pesticides, as well as significant reductions with contaminants associated with suspended sediment. Results of this study will corroborate the efficacy of floating vegetation for water treatment, and demonstrate the efficacy of GAC treatment in a large-scale field application.

### TP080 Comparing relative efficacies of granulated activated carbon and biochar to reduce pesticide loading using laboratory bench scale experiments

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Granulated activated carbon (GAC) has been shown to reduce the pesticide load in simulated agricultural runoff. Biochar is a potentially more cost-effective and convenient alternative to GAC, but little is known of its pesticide sorbing capabilities. This study looked at the efficacy of GAC and biochar to reduce the concentration of commonly used pesticides, chlorpyrifos and imidacloprid. Experiments proceeded in two phases, each designed to simulate sixteen days of irrigation runoff events. Scaled-down experiments represented total irrigation flows of approximately 100,000 liters. The first phase treated a 1 ppb chlorpyrifos stock with individual columns of GAC, biochar, or a combination of both materials. Chlorpyrifos concentrations of the stock and post-column samples were measured daily using enzyme-linked immunosorbent assays (ELISAs). Every four days, post-column samples were analyzed for chlorpyrifos using gas chromatography/mass spectrometry (GC/MS), and for toxicity using 96-hour acute *Ceriodaphnia dubia* bioassays. Phase two was conducted with 5 ppb imidacloprid stock and fresh columns. Column breakthrough of imidacloprid was monitored every four days analytically with GC/MS. Toxicity from column breakthrough was monitored using

10-day *Chironomus dilutus* bioassays. No chlorpyrifos breakthrough was observed with ELISA measurements or toxicity. Preliminary results for the imidacloprid experiment show no post-column toxicity. Analytical chemistry results for both experiments are pending. These laboratory experiments demonstrate that biochar is a reasonable alternative to GAC for the treatment of agricultural pesticides, but field trials are necessary to confirm large-scale effectiveness.

**TP081 Mechanisms of pesticide removal from agricultural tile drainage: An assessment of woodchip bioreactor performance**

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The discharge of pesticides via agricultural drainage and runoff is a topic of increasing concern, as many of these compounds pose risks to human and environmental health. Many registered pesticides have been classified as acutely toxic, carcinogenic, and/or endocrine disrupting, and other pesticide products are employed without full knowledge of their potential toxicity. Moreover, certain chemical classes of pesticides are persistent in the environment, and some more mobile in aqueous systems, making their removal from water at the point of application desirable. Woodchip bioreactors have been recognized for their efficacy in nitrogen removal from agricultural runoff and may also provide a cost-effective method for removal of pesticides from tile drainage. Preliminary field studies demonstrated high removal of neonicotinoid and pyrethroid pesticides across woodchip bioreactors during summertime operation. The present study assesses woodchip bioreactors at laboratory and field scales for the joint removal of nutrients and pesticides from tile drainage. First, temporal and spatial removal patterns of field woodchip reactors (average hydraulic residence time 20-24h) are evaluated. Pesticide removal is characterized using both liquid and gas chromatography time-of-flight mass spectrometry, with targeted and suspect screening workflows to cover a broad range of compounds. Based on field-scale results, lab-scale reactors are constructed to identify and characterize the removal mechanisms for specific compounds and selected degradates of interest, representing major chemical classes of pesticides. These compounds include fipronil, thiamethoxam, imidacloprid and clothianidin. Variables of interest during the lab-scale study include temperature, woodchip particle size, contribution of microbial activity, dissolved oxygen concentration, and hydraulic residence time. This work helps to identify primary mechanisms for joint pesticide removal in order to optimize designs for woodchip bioreactors.

**TP082 Evaluating Consumer Behaviors to Support Refinement of Pesticide Use Assumptions in EPA's Outdoor Residential Screening Model**

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The USEPA recently submitted screening-level residential model scenarios to assess exposure for outdoor consumer products as part of periodic pesticide registration reviews. The assumptions and scenarios in the screening residential models may not be characteristic of consumer use practices in terms of amounts applied, treatment sites and areas, and frequency of use. Consumer use of residential pesticide products may be sufficiently different from professional use practices that the two categories should not be evaluated with the same model scenarios in a combined risk assessment. Evaluation of consumer use practices as professional may result in overestimation of use and alter the risk assessment outcomes for outdoor residential consumer products. To refine consumer application assumptions for a typical household lot, including treatment amounts, site types, site areas and timing, we used information from consumer use surveys. Additional survey information provided refinements to timing and proportion-of-use assumptions at the watershed scale. Geographic differences in consumer product consumption associated with differences in pest pressure also provide evidence for the value of regional adjustments to application assumptions. The proposed refinements based on consumer use surveys provide realistic adjustments to the screening outdoor residential model, which may be of value during the registration review process.

## Persistence and Biodegradability Assessment

**TP084 Assessments of Biodiesel and Petrodiesel Impact on Soil Microbial Communities**

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Organic pollutants could pose long-lasting impacts on the environment due to their low biodegradability. Understanding the effect of organic chemicals on microbial communities will improve not only the regulatory evaluation of environmental risk but also our understanding of biodegradation of organic compounds. Biodiesel has been considered as a viable substitute for petrodiesel mainly because it is readily degraded. However, very limited studies have examined biodiesel and petrodiesel impacts on soil microbes, and whether biodiesel is more microbial-friendly than petrodiesel is unknown. This laboratory study compared the effects of petrodiesel and three types of biodiesel on soil microbial communities in sandy-loam soil. The soil was manually contaminated with three types of biodiesel (castor ethyl ester, castor methyl ester, and safflower methyl ester), petrodiesel, and distilled water (uncontaminated control) at 2% by weight. During a 180-day incubation period, samples were collected for residual analysis and microbial 16s sequencing. GC-FID was used to detect the chemical residuals, and half-life of petrodiesel and biodiesels were calculated based on the exponential decay model. Soil microbial community composition and function were analyzed based on 16s sequencing results where potential biomarkers were identified for each contaminant. Results demonstrated that even though biodiesel showed low persistence in soil than petrodiesel, biodiesel and petrodiesel imposed similarly irreversible impacts on soil microbial communities. Biodiesel treatments decreased the diversity and ecological functions of microbial communities more than the petrodiesel treatment, and distinctive biomarkers were found in different treatments. In conclusion, our results suggested that biodiesels should not be automatically considered as harmless substitutes for petrodiesel.

**TP085 Fate of emerging micropollutants and mercury in Capbreton Submarine Canyon sediment in controlled experimentations (Biscay Bay, SW France)**

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Priority substances set by the Water Framework Directive (WFD) are of major interest to evaluate the quality of coastal and marine systems, the final receptors for pollutant emissions. Emerging substances not regulated by the WFD, i.e. personal care products and some pharmaceuticals, are of high concern since only scarce information of their occurrence, reactivity and impact are available in the deep sea sediments. In this work, transformation kinetics of mercury species (IHg,MMHg), sunscreen (ODPABA) and synthetic musk (HHCB) were studied independently from three Capbreton Canyon sediment stations according to the distance to the coast. Slurry incubations were spiked with enriched isotopic mercury species, with ODPABA and HHCB to determine methylation, demethylation potentials and degradation potentials, respectively. Those experimentations were performed under biotic/abiotic, oxic/anoxic conditions in triplicate. Mercury species analysis and emerging micropollutants analysis were performed for each kinetic times (0,1, 2, 4, 7, 11, 14 days and 0, 5, 12, 39, 50, 81, 110 days for Hg and emerging micropollutants, respectively). Taxonomic diversity (16s DNA, MiSEQ) were analysed for initial and final kinetic times to determine microorganisms diversity.

Then, microorganisms were isolated by dilution and enrichment in anaerobic and aerobic conditions. Net methylation potential were only observed under biotic conditions revealing the involvement of prokaryotes in the fate of Hg species. This was confirmed by taxonomic diversity and sequencing of *hgcA* gene, a proxy for biotic methylation. Two main families of methylating bacteria have been identified, *Desulfobulbaceae* and *Desulfobacteraceae*, suggesting their involvement in the biotic methylation process. Higher methylation potential was observed for the coastal station whereas demethylation potentials were similar for the three stations suggesting that environmental parameters (e.g. input of continental organic matter) also control methylating microbial activities. Degradation potential was only observed under biotic condition for ODPADA and fungi have been isolated. These results seem to confirm the role of the Capbreton submarine-canyon to trap, to transfer and to transform micropollutants. Half life times determination reveasl the differential resilience of such deep sea sediment for some priority and emerging substances and the key role of microorganisms.

**TP086 Lowering of the Concentration Range of the Ready Biodegradability Method OECD 301B**

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The OECD 301B Guideline '$CO_2$ Evolution Test' also known as the 'Modified Sturm Test' has been a trusted method in determining if a test substance is 'readily biodegradable'. If a test substance exposed to a dilute inoculum generally comprised of activated sludge achieves 60% biodegradation equivalent to 60% of its organic carbon being converted to $CO_2$ within a 10-day window (once reaching 10% $CO_2$ evolution) during the 28-day test, the test substance can be labeled 'readily biodegradable'. However, based on the method in its current form with starting concentrations at 10 mg/L as carbon, some compounds that could potentially be biodegradable may fail this test if they are inhibitory at this relatively high concentration which may be above many compounds' Predicted Environmental Concentrations (PEC). A 301B study design was modified in this experiment by conducting the prescribed test with one of the standard reference substances, sodium benzoate, but at lower than recommended concentrations. This was conducted to determine the sensitivity of the test method at much lower and more environmentally relevant concentrations. The concentrations used in this experiment are 10, 5, 2 and 1 mg/L as carbon. The results will demonstrate if the method is appropriate at concentrations of 1/2, 1/5th and 1/10th of the lowest test concentration of 10 mg/L carbon currently recommended in the OECD 301B Guideline.

**TP087 Soil bacterial community composition is altered after the degradation of estrone and triclosan mixture**

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Wastewater derived from domestic use commonly contains mixture of pharmaceutical and personal product (PPCP), but its persistence and accumulation in the soil and the consequences for soil microbial community processes are poorly understood. Estrone and triclosan are two common PPCP of domestic wastewater. Soil microbial communities degrade a variety of PPCPs however; most studies have only addressed single compound designs neglecting the reality of their co-occurrence in nature. In this study, we examined the interaction between estrone and triclosan mixture, their potential to persist and disrupt soil microbial community function and how the microbial community react in pre-exposed soils and naïve soil. Soil with previous exposure to these chemicals (conditioned soil) and soil not previously exposed (unconditioned soil), was spiked with estrone, triclosan, and a 1:1 mixture of estrone: triclosan, and incubated for 90 days in the dark at 27°C. We examined soil microbial function dynamics using commercial Biolog EcoPlates™. Microbial degradation rates were compared over the 90 days' incubation period using high performance liquid chromatography. 16S rRNA analysis to determine changes in microbial community over time. There was significant increase in substrate activity and substrate richness in all treatments. Each microbial community utilized different carbon substrates by day 90 whereas they had exhibited similar substrate utilization at day 0. Estrone and triclosan showed the same pattern of biological degradation in both conditioned and unconditioned soils, exhibiting half-lives of 5.9-6.8 days (estrone) and 24.1-26.9 days (triclosan). The rate of degradation of the estrone:triclosan mixture was the same as the individual compounds. There was a decrease in species diversity between control at day 0 and other treatments at day 90 with establishment of unique OTUs in each treatment group at day 90. Bacillus sp. was dominant in all the day 90 treatments. Soil microbial communities can degrade estrone and triclosan in the soil thus preventing accumulation in soil and subsequent contamination of ground water.

**TP088 What's in your laundry detergent? An evaluation of common surfactants found in laundry detergents and their PBT characteristics**

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In the past, high performance surfactants such as nonylphenol ethoxylates (NPEs) were widely used in commercial laundry operations. California Department of Toxic Substances Control (DTSC) recently developed a Product-Chemical Profile on NPEs in laundry detergents under the framework of its Safer Consumer Products Regulations. This framework identifies specific product-chemical combinations that have the potential to expose people or the environment to a harmful hazardous chemical. When released into the environment, NPEs and their degradation products can persist and accumulate in the aquatic environment, potentially causing harm to fish and aquatic invertebrates, and impair wildlife growth, reproduction, development, and survival. A voluntary industry phase-out of NPEs was initiated in 2010, with the goal of complete phase-out from all detergents by 2014. Although chemical alternatives may be used in lieu of NPEs, it is important to characterize potential adverse impacts to people and/or the environment. We surveyed common household laundry detergents to evaluate the chemical alternatives of NPEs and their effect on human health and the environment according to persistence, bioaccumulative, and toxicological (PBT) properties.

## Integrating Big Data into the Bigger Picture

**TP089 A Multi-omic Approach to the Analysis of Organism Responses to Great Lakes Sediment, Effluent and Surface Water Exposures**

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Sediment, effluent and surface water samples were collected from various sites across Hamilton Harbour, Toronto Harbour, Humber Bay and Lake Erie between 2014 and 2015. Larval Hexagenia sp. and sexually immature rainbow trout were exposed to these samples in the laboratory for 48 hours. Liver, plasma, and fin tissue were collected from exposed and control rainbow trout while exposed and control Hexagenia were collected whole. Levels of 11 transcripts in rainbow trout and 7 in Hexagenia were measured. Shotgun proteomics data were generated for Rainbow Trout and Hexagenia. Two hundred and nineteen metabolites including amino acids, lipids, bile acids, and fatty acids were quantified. A total of over 500 individual contaminants and water quality indicators were measured in the effluent, surface water, and sediment samples. Results showed distinct signatures by exposure type. Metabolomics data in Hexagenia exposed to sediment from Hamilton Harbour sites with high persistent organic pollutant concentrations showed 60 metabolites differing significantly between sites and correlating with contaminant levels. Two transcripts also showed statistically significant differences between these sites. One hundred and one Hexagenia proteins had significantly different levels following exposure to Hamilton Harbour effluent samples. It is expected that the combination of 'Omic results will have higher differentiating power than data from a single endpoint measurement. However, this may not be so for rainbow trout which were generally less responsive than Hexagenia – possibly due to differences between these two species and their relative levels of exposure.

**TP090 Untangling Adverse Outcome Pathway Networks: Extracting, Benchmarking, and Applying Emergent AOP Knowledge**

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As the community of toxicological researchers and risk assessors adopt the adverse outcome pathway (AOP) paradigm for organizing toxicological knowledge, the number and diversity of AOPs in the online AOP knowledge base (KB) continue to grow. To characterize this growth, we assembled all AOPs in the KB into a network and measured several benchmarking properties including basic summary information, and network structure and connectivity metrics. We then identified and quantified the emergence of new knowledge that resulted from combining all AOPs into a single network. We found that networking the 187 user-defined AOPs described in the KB resulted in the emergence of 9405 unique, previously undescribed, linear AOPs (LAOPs). An AOP-KB growth simulation was used identify factors than influenced the rate of new LAOP emergence. We found that the creation of new AOPs that borrowed components from previously existing AOPs in the KB was the most relevant factor for the emergence of LAOPs, but that the introduction of non-adjacent key event relationships and feedback cycles were also important factors. Finally, we provide examples of how to identify application-specific paths of interest from this large number of LAOPs using a weighted path approach. These results show that the AOP-KB network may have considerable value as a source of emergent toxicological knowledge. These findings are not only helpful for understanding the nature of this emergent information, but can also be used to manage and guide future development of the AOP-KB, and to tailor AOP information to specific risk assessment and research applications.

## The Use of Historical Control Data in Ecotoxicological Study Assessments

**TP091 A Review of the Time to Hatch Endpoint in Fish Early Life-Stage Studies with Fathead Minnow**

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Early development is considered an especially sensitive stage in the fish life-cycle. As a result, many regulatory agencies recognize the fish Early Life-Stage (ELS) test as an important component of environmental risk assessments. The most recent revision of EPA guideline OSCPP 850.1400 updated the list of recommended endpoints for the fish ELS to include mean time to hatch. This endpoint represents the mean elapsed time between test initiation and completed hatch for the embryos in a replicate. Hatch observations are typically performed once daily, along with other biological observations. This endpoint is thought to be most suitable in fish ELS studies with species that exhibit hatching behavior over a longer hatch period (i.e., rainbow trout, the recommended freshwater fish species in this guideline), but the relevance of the endpoint is less understood in test species with shorter hatch periods. This review examines the control mean time to hatch for ten fish ELS studies conducted with fathead minnow (*Pimephales promelas*) since the implementation of OCSPP 850.1400 (2017 – 2018). Further, analyses were performed on complete study data sets to determine if the mean time to hatch endpoint could be correlated to subsequent fish ELS endpoints (i.e., size; measured as total length and/or either wet or dry weight, and post-hatch success) to indicate whether mean time to hatch is a relevant predictor of subchronic toxicant related effects. Review found that mean time to hatch across the ten studies was 3.5 days (S.D. 23%). On an individual organism basis, 31.6% of organisms hatched by test day 3, 68.0% hatched by test day 4, and 0.5% hatched by test day 5. Correlational analyses on whole study data sets found that there was no correlation between the mean time to hatch endpoint and any of the other subsequent endpoints in 7 of the 10 studies examined. The remaining three studies showed significant correlations between mean time to hatch and the size and post-hatch success endpoints. However, all but one test showed a stronger correlation between post-hatch success and size than between those endpoints and mean time to hatch. The remaining study demonstrated strong statistically significant effects in the size endpoints as well as mean time to hatch. These results raise the question of whether the mean time to hatch endpoint as measured is suitable as a predictor of biologically relevant subchronic toxicant related effects in less extreme data sets.

**TP092 An Examination of Historical Control Data and Endpoint Sensitivity for Tier I Honey Bee Laboratory Studies**

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Tier I laboratory honey bee testing as defined by the Environmental Protection Agency (EPA) Office of Pesticide Program include test methods that are described by either draft or recently finalized OECD guidance. These tests include the larval acute oral exposure (OECD 237 Guideline), larval chronic oral exposure (OECD 239 Guidance Document) and adult chronic oral exposure (OECD 245 Guideline). Smithers Viscient has begun to compile data for the Tier I testing as a result of the recent influx of data-call-ins issued by the EPA for agrochemical product registration that require information from these exposures. In this poster, historical control data is presented and compared against acceptability criteria. As these test methods are rather novel relative to other ecotoxicity testing, control performance will also be tracked over time to assess if laboratory experience is a factor in achieving acceptable results. In addition, trends in endpoint ($LD_X$/$ED_X$) sensitivity will be examined across the three tests types to define any correlations in terms of toxicity. The growing database and experience with Tier I laboratory honey bee testing may be utilized to highlight any potential redundancy in the testing strategy (single dose larval acute and repeated dose larval chronic) or increase the ability to better predict toxicity using the results of a single test type.

**TP093 New Daphnia magna Historical Control Data Needed to Evaluate Chronic Study Results**

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Historical control data (HCD) is useful to support results of a study when a data set may be unclear due to high or low control variability. The reproductive endpoints for *Daphnia magna* chronic testing have changed recently with the finalization of the OCSPP 850.1300 guideline. Additional response variables are now required including $F_0$ production rate of first brood release and number of live offspring per $F_0$ adult per reproductive day. Since collection and calculation of these response variables only began after the 850.1300 guideline was finalized in October of 2016, the historical database for these endpoint requirements is limited. Additionally, the statistical evaluation of the multiple reproductive endpoints can lead to conflicting results within a single data set. Daphnid length data typically has low variability and many years of data collection has provided an extensive historical control database. Since this endpoint demonstrates low variability, it is common for slight differences in daphnid length to be significantly different when compared to the control while still remaining within historical ranges and likely not biologically relevant, especially at a population level. Since low variability can cause statistical analysis to become overly sensitive, the extensive historical database for length data can assist in such cases to make an argument for adjustment to the statistically determined NOEC value based on these historical data values. The recently required daphnid reproduction response variables also have low variability in the data sets collected so far, especially the production rate of first brood endpoint. Based on the daphnid life cycle, brood release is typically within a two-day period and with once daily observations, this endpoint does not provide a true biological difference unless outside of the historical range. Using the daphnid length data as a model, gathering more historical control data on the production rate of the first brood endpoint will likely prove to be helpful in instances where low variability yields uncertain or unreasonable endpoint results.

## Chemical Mixtures in Urban Systems – Screening and Prioritization of Emerging Contaminants

**TP094 Identification of emerging micropollutants in effluents from WWTP, Korea via suspect and nontarget screening**

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Over the last decades, many environmental pollutants such as pharmaceuticals, personal care products, and pesticide have been studied in water system. However, there are many other substances that are still unknown and/or excluded from the target list for chemical analysis. To identify them, suspect and nontarget screening have been applied using LC-HRMS analyzing accurate mass. In the present study, we tentatively identify emerging micropollutants in effluents which have never reported or less information in Korea. The effluent samples were taken respectively at the 7 WWTPs, which discharge effluent to the Nakdong River basin in September, 2016 and analyzed via suspect and nontarget screening using LC-HRMS (QExactvie+ Orbitrap). As the results, 14 pharmaceuticals (e.g., amisulpride, losartan, niflumic acid, etc), 5 metabolite (e.g., 2-amino-6-methylmerca albendazole sulfone, etc), 2 herbicide (e.g., ametryn, atratone), 3 Etc (e.g., 4-methylbenzotriazole, tributyl citrate acetate, tributyl citrate) were tentatively identified in the effluent samples. 4-methylbenzotriazole used as a corrosion inhibitor was showed the highest intensity. 4-methylbenzotriazole, niflumic acid (drug used for joint and muscular pain) and tributyl citrate acetate (plasticizer) were detected the highest frequency. Dihydrocodeine, an opioid narcotic analgesic was identified in 4 effluent. Valsartan acid was identified in the 6 effluent. Of the tentatively identified 24 compounds, amisulpride, fexofenadine and losartan were confirmed with reference standard. Confirmation and quantitative analysis of the remained substances is currently under study.

**TP095 Identifying and assessing the toxicity of transformation products formed during oxidative water treatment**

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Oxidation processes used for water treatment transform many commonly detected organic environmental contaminants. It is often assumed that “removing” the contaminant molecules will reduce the toxic effects that may be caused by their presence. However, transformation products can also have biological effects and in some cases their toxicity exceeds that of the parent molecule. Even a single compound can produce a complex mixture of transformation products, and determining ways to evaluate which mixture components are potential causes of toxicity is an important step in assessing contaminant removal systems and prioritizing contaminants for removal. We studied personal care product ingredients that are frequently detected in wastewater treatment effluent and swimming pools, and identified their transformation products using high resolution mass spectrometry. Additionally we measured adducts formed from reactions between transformation products and biomolecules as a way to identify transformation products and pathways that may have unintended negative effects on organisms.

**TP096 Profiling and Identification of Poly- and Perfluoroalkyl Substances (PFAS) in Wastewater Treatment Plant (WWTP)**

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The current and historic usage of aqueous film-forming foam (AFFF) at military and civilian sites have drawn much attention in recent years due to possible linkage to poly- and perfluoroalkyl substances (PFASs) contamination in water systems. Recently, PFASs were measured at relatively high levels in effluent in some San Francisco Bay Area wastewater treatment plants that were impacted by AFFF usage. However, PFASs identified using targeted analysis methods are only a small portion of the PFAS known to be present in AFFF formulations. As a follow-up study, high resolution mass spectrometry (HRMS) was commissioned to investigate the PFASs at an airport wastewater treatment plant during an AFFF introduction event. AFFF-derived PFASs and their transformation products were identified using high resolution quadrupole time of flight mass spectrometry (QTOF/MS). Using a combination of targeted and non-targeted analysis (NTA) approaches, molecular features that were extracted from the raw total scan chromatography were screened, prioritized and tentatively identified with compounds from an in-house PFAS database. For prioritized features that have no database match, chemical formulas were generated based on exact mass, isotope distribution and isotope spacing. We preliminarily identified the presence of various PFASs known to be present in fluorotelomer-based AFFF formulations, including 6:2 fluorotelomermercaptoalkylamido sulfonate (FTSAS), 6:2 fluorotelomer sulfonamide alkylbetaine (FTAB), their homologues from 4:2 to 12:2, as well as various transformation products, primarily derived from the biological oxidation of 6:2 FTSAS within the treatment plant. We identified novel PFASs, proposed their structures and possible transformation routes. Optimized working flow with a focus on PFASs were proposed. The unknown screening approach combined with the profile comparison (influent vs. effluent) is critical in establishing PFAS transformation intermediates and products, their fates in the wastewater treatment plant, and assessing potential impact to the Bay water system.

**TP097 Pharmaceutical of emerging concern in urban wastewaters from Costa Rica: The case of Levofloxacin, Atenolol and Sulindac using oDGT passive sampling**

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The occurrence of the fluoroquinolone Levofloxacine, b-blocker Atenolol and NSAID Sulindac was evaluated in the urban wastewaters from the Great Metropolitan Area in San José, Costa Rica. Sampling sites were located in a major hospital, the most important wastewater treatment plant of the city and the river highly polluted of the area. Passive sampling using oDGT technology was applied by allowing continuous samplers in each site for a 15 day period during 2017. The quantitative analysis was performed using liquid chromatography-electrospray ionization tandem mass spectrometry. According the environmental concentrations (TWA) found, maximum concentrations of 1853 ng/L for Levofloxacin and 532 ng/L for Sulindac, were detected in the hospital effluent, while 3948 ng/L was for Atenolol in the treatment plant. Results corroborated that primary and secondary treatment facilities don´t reduce the contamination for the studied drugs. Due to the tropical climate of Costa Rica, direct photolysis was studied as a principal fate process, through the photochemical property of the quantum yield (φ) and the pH influence, finding values of $\varphi$=0,101 for Levofloxacin and $\varphi$=0,0791 for Sulindac at pH 7. These results show the potential risk that could affect to the aquatic ecosystems and the general public exposed, and could help to the health and environmental authorities, to consider investments in advanced technologies for the treatment of the wastewaters in the country. Key words: pharmaceuticals, Levofloxacin, Atenolol, Sulindac, emerging contaminants, urban wastewaters, Costa Rica

**TP098 Emission characteristics of Dioxins and HCB, PeCBz of MSWs prevention facility in the Republic of Korea**

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Introduction : Total generation amount of the municipal solid waste in Korea is steadily on the rise, according to literature sources and representative removal method of this waste is incineration. In case of Municipal solid waste incinerators(MSWs), it could occur harmful substance being unintentionally produced Persistant organic pollutants(POPs) including Dioxins(PCDD/Fs), Hexachlorobenzene(HCB) and pentachlorobenzene(PeCBz). In this study, we analyze POPs(PCDD/Fs, HCB, PeCBz) concentration from stack through prevention facility focused on MSWs and check correlation of unintentional PCDD/Fs and HCB, PeCBz. and a composition of each prevention facility was classified to 4 group(A,B,C,D). Group A is CC→WHB→SDR→BF→STACK, B is CC→SNCR→WHB→SDR→BF→Stack, C is CC→WHB→SDR→BF→SCR→Stack. D is included to BF→WS process. *CC:Combustion Chamber, WHB:Waste Heat Boiler, SCR:Selective Catalytic Reduction, SNCR:Selective Non Catalytic Reduction, SDR:Semi Dry Reactor, WS : Wet Scrubber <strong>Materials and Methods : Sampling was proceeded from 2014 to 2016 at concentration of PCDD/Fs, HCB, PeCBz. The measurement and analysis of them formed based on the POPs official standard. All particulate and gaseous matter collected by stack sampler(KXC-572, APEX) were extracted by soxhlet extraction with toluene over 17hours then subjected to a series of clean-up columns including acidic multi-layer silica gel columns and activate basic aluminum oxide column. HCB and PeCBz conducted only multi-layer silica gel columns. After reference satandard substance was injected to final concentration solution, it was analyzed with HRGC/HRMS for PCDD/Fs and HRGC/LRMS for HCB&PeCBz Results : The concentration correlation between Dioxins and HCB, that between Dioxins and PeCBz in a variety of waste type(industrial, municipal, specific and medical waste) results in the insignificant relationship except for MSWs. Facility of C set to after SCR indicates low average concentration of all POPs( PCDD/Fs, HCB, PeCBz) and is 0.003 ng/Nm3, 0.000 ng/Nm3, 0.032 ng/Nm3 respectively. In the case of D group included WS, It is relatively higher than these group and concentration of POPs (PCDD/Fs, HCB, PeCBz) is 0.504 ng/Nm3, 32.949 ng/Nm3, 120.883 ng/Nm3 respectively. This suggests that prevention facility using SCR process provided the better efficiency and which using WS process could cause decline efficiency of the removal of PCDD/Fs.

**TP099 Comprehensive nontarget analysis of sediment and aqueous environmental samples via SPE LC-ESI-HRMS: A case study for the Florida Everglades**

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The Contaminant Assessment and Risk Evaluation (CARE) Project was an extensive study that aimed to assess and inform resource managers about risks to the ecosystems of Everglades National Park, Biscayne National Park, and Big Cypress National Preserve. The analyses for the project included organochlorine pesticides, trace metals, and contaminants of emerging concern, such as pharmaceuticals and personal care products. Recently, citizen complaints and public reports of potential contamination near Everglades City and Chokoloskee Bay have renewed the interest in assessing the current conditions in the bay and nearby coastal basins. To this end, sediment and water samples were collected seasonally from six sites across the transect of the Barron River through Everglades City, Florida, and a method for the comprehensive nontarget analysis of aqueous and sediment samples was developed. Water samples were analyzed for environmental parameters on site and bulk organic matter characterization by fluorescence before nontarget analysis via an online solid phase extraction liquid chromatography coupled to an electrospray ionization-high resolution mass spectrometer (SPE LC-ESI-HRMS). Compared to previous reports, a different approach in terms of sediment sample preparation was performed, where samples were sequentially extracted by accelerated solvent extraction (ASE) with water, methanol, and acetonitrile before HRMS analysis of each fraction. Nontarget data interpretation was undertaken on Thermo Scientific's Compound Discoverer 3.0 using a routine workflow for identification of unknown environmental contaminants. Initial data assessment shows an increasing chemical diversity as sampling moved along the north-south transect from natural waters to an urban environment, with primarily natural products and some human tracers detected at northern sites, with the introduction of plasticizers, surfactants, and various pharmaceuticals appearing in increasing variety in southern sites.

**TP100 There must be something in the water: Non-targeted assessment of emerging contaminants in North Carolina drinking water sources**

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Discovery of the emerging polyfluorinated alkyl substance GenX at elevated concentrations in ambient waters and finished drinking waters of the Cape Fear region in North Carolina in 2016 has led to increased awareness of the problems surrounding emerging organic contaminant occurrence in drinking water sources in the State. We have conducted periodic water sampling at drinking water intakes located in the Neuse, Haw, and Catawba river basins in North Carolina for subsequent emerging pollutant reconnaissance. These sites span the range of industrialized (Haw), urbanized (Catawba), and agriculturally-dominated (Neuse) watersheds, reflecting multiple land uses and potential emerging pollutant sources to drinking water sources. Samples were analyzed using a novel LC-HRMS/MS based workflow, with data acquisition conducted on an Orbitrap Fusion tribrid MS and compound detection, prioritization, identification, and functional use classification performed using custom cheminformatic and computational scripts. Our results indicated a complex mixture of natural and anthropogenic organic compounds in NC surface waters, with analytical signals detected for > 5,000 discrete compounds, and high-confidence tentative structural ID's for > 300 of these. The tentatively identified compounds present at high relative intensity included markers of wastewater (e.g. sucralose, methylbenzotriazoles, and pharmaceuticals), stormwater (e.g. benzothiazole and substituted urea and guanidine vulcanization accelerants), and agrochemical input (e.g. simazine, atrazine, and fungicides such as carbendazim). In general, the urbanized and industrialized watersheds were dominated by stormwater markers and wastewater-derived contaminants, while the rural, agricultural watersheds appeared to show elevated levels of pesticides and other crop management chemicals, consistent with apparent land use. Finished drinking waters were enriched in apparent oxidative transformation products and natural products relative to raw waters, consistent with formation during drinking water disinfection. In some cases finished drinking waters collected from newly constructed buildings showed evidence of elevated plasticizers and other polymer additives, possibly leached from new water delivery infrastructure in the buildings. Overall, our results illustrate occurrence of diverse emerging contaminants in raw and finished drinking waters within the state of North Carolina.

## Linking Mechanism of Action to Physiological and Ecological Effects in Aquatic Species

### TP101 Estrogenic potential of Cyclohexylphenylketone as mediated through metabolic activation in rainbow trout

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Phenones and hydroxy-benzophenones are widely used as UV radiation filters, and in the manufacturing of insecticides and pharmaceuticals. Understanding the estrogenic potential of these chemicals is of interest to the US Environmental Protection Agency (USEPA) and the international community. The current study sequentially combined complementary in vitro rainbow trout estrogen receptor (rtER) binding and liver slice vitellogenin (Vtg) mRNA induction assays in the context of a defined ER-mediated Adverse Outcome Pathway (AOP). Cyclohexylphenylketone (CPK), which did not bind to the rtER in cytosol, was biotransformed within liver tissue to one or more metabolites that induced Vtg expression. In absence of Standards, nine CPK metabolites were identified by combining gas chromatography-mass spectrometry (GC-MS) with and without derivatization, liquid chromatography (LC) with tandem MS, and LC with high resolution time of flight (ToF) MS spectra information. LC fractionation showed relationships between LC- and GC-MS data needed to assess substitution patterns. Results supported that CPK is primarily metabolized through phase I oxidation of the cyclohexyl ring and not the phenyl group as anticipated. Two metabolites (M1 and M2; MW 186) were analyzed by GC-MS and enzyme conjugation studies and proposed to be cyclohexenyl-derivatives. M6-M9 were confirmed using derivatization techniques as hydroxylated metabolites (MW204), with the potential for undergoing phase II conjugative metabolism to glucuronides and sulfates. Lastly, M3, M4 and M5 were identified as cyclohexanone-derivatives of CPK (MW 202), resulting from the redox interconversion of the hydroxylated metabolites. Information obtained allowed the proposal of a novel metabolic pathway and potential adverse effect mechanism for cyclic phenones in rainbow trout liver slices.

### TP102 Cholinesterase inhibition by sublethal exposure of organophosphate pesticides can reduce the ability of a tropical freshwater fish to escape predation

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The continuous discharge of pesticides from agricultural activities have negative effects on aquatic organisms and ecosystems. Highly toxic pesticides like organophosphates have low persistence in the environment but are applied very frequently in crops, becoming an eminent threat for aquatic ecosystems. Biomarkers are use in ecotoxicology to study toxicant effects at low concentrations of exposure. However, most sublethal studies examined biomarkers at low levels of biological organization, mainly cellular, which lack evident ecological relevance. In order to understand the ecological implications of such effects it is necessary to evaluate responses at different levels of biological organization (sub-individual, individual level), and study their interactions. We used a multibiomarker approach to estimate the toxic effects of ethoprophos, an organophosphate insecticide commonly used in banana plantations, on the tropical fish *Astyanax aeneus* (Characidae). We measured biomarkers at sub-individual (cellular) and individual (metabolism, behavior) levels and examined cause-effect relationships among these responses. Exposed fish showed a significant inhibition of brain Cholinesterase (ChE) enzyme activity (57%), reflecting the high pesticide neurotoxicity. However, other biomarkers like oxidative stress (CAT, LPO), biotransformation reactions (GST), and resting metabolic rate were not affected by the pesticide. Antipredator behaviors such as predator avoidance and risk assessment (light/dark preference) were modified by the pesticide exposure. Exposed fish escaped slower from a simulated predator attack and showed preference for brighter areas in a novel tank, changes that might increase predation vulnerability. Escape time was negatively affected by ChE activity, suggesting that pesticide-induced cholinesterase inhibition increases escape time. Ethoprophos caused disruption of essential cellular processes, leading to behavior impairment which might cause a detriment in the fish ecological performance. These results provide evidence that impacts of organophosphates pesticides on fish population can occur even at short exposures at very low concentrations. By using a mechanistic approach, individual responses can be scaled up to population effects, improving the estimation of pesticide effects on wildlife and giving a higher predictive ability to risk assessment studies.

### TP104 Bifenthrin as an endocrine disrupter: Linking the mechanism of estrogenic effects of bifenthrin exposure to sex determination alteration in zebrafish

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Bifenthrin is a commonly used pyrethroid insecticide, and a known endocrine disrupting compound. Bifenthrin has been shown to have estrogenic effects in fish, eliciting increases in plasma 17-β-estradiol (E2) and upregulation of genes downstream of estrogen receptors (ERs) including *vitellogenin*. Further, our preliminary data suggest bifenthrin can alter sex determination in wildtype zebrafish. Because bifenthrin has been shown in cell culture to bind to and activate ?estrogen receptors, and elicit estrogenic responses in vivo, it has been assumed that ?bifenthrin acts as an ER agonist in vivo.? However, due to the presence of endogenously produced estrogen confounding a primary vs. secondary effect, the direct activation of ERs by bifenthrin has yet to be confirmed in vivo. The goal of this project is thus to determine in vivo if bifenthrin directly activates ERs. To measure ER activation directly, as opposed to downstream events, transgenic estrogen receptor reporter fish were used. Tg(*5xERE:egfp*) fish produce green fluorescent protien (GFP) driven by estrogen response elements. Tg(*5xERE:egfp*) fish were exposed to bifenthrin at environmentally relevant concentrations as either larvae or juveniles, and evaluated for GFP mRNA abundance via quantitative polymerase chain reaction (qPCR). Consistent with previous findings that bifenthrin is estrogenic in juveniles and anti-estrogenic in larvae, bifenthrin was found to increase relative GFP in juveniles by 1.4 log2 fold-change at 10ng/L, and slightly decrease relative GFP in larvae by 0.3 log2 fold-change at 10ng/L, as well as alter estrogen receptor and cytochrome expression. These results support that the Tg(*5xERE:egfp*) fish can be used to measure the endocrine disrupting effects of bifenthin at environmentally relevant concentrations. Additionally, we show ER activity in response to bifenthrin exposure in the absence of endogenously produced estrogen by using null mutants for the aromatase responsible for estrogen production. Using zebrafish as a model species, the results reveal valuable knowledge of endocrine disruption mechanism linked with physiological outcome applicable to wild fish populations and humans at risk from exposure to this commonly used insecticide.

**TP105 Use of CRISPR/Cas9-mediated gene disruption in Xenopus tropicalis to evaluate thyroid-related targets of toxicological concern**

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CRISPR/Cas9-mediated gene editing is now an easily employed straightforward tool for studying gene function and has logical applications in the field of toxicology. Functional knockout of a protein implicated as a toxicological target serves as a highly specific method to evaluate downstream biochemical and apical effects when a molecular target is perturbed – in lieu of model chemical exposures. This approach could prove most useful for molecular targets that are not yet well-characterized by traditional toxicological approaches involving exposures to model chemicals or for targets where model chemicals are unidentified. To demonstrate the effectiveness of gene editing for thyroid disruption research, we employed the CRISPR/Cas9 system to knock out the thyroperoxidase (TPO) gene in model amphibian species *Xenopus tropicalis*. Newly fertilized embryos were microinjected with CRISPR/Cas9 ribonucleoprotein (RNP) complexes designed to target three different sites within the TPO gene. The viable larvae for each of the RNP-injected, non-injected and mock-injected groups were grown out in flow-through tanks for continued evaluation. All of the surviving non-injected controls and mock-injected larvae completed metamorphosis with a median metamorphosis time of 48 days (n = 85 and 42, respectively). Of the surviving RNP-injected larvae, 70 out of 89 completed metamorphosis with a median metamorphosis time of 48 days while 19 putative knockouts were arrested at Nieuwkoop and Faber stage 55 (onset of pro-metamorphosis). This arrested development phenotype was consistent with previous studies of TPO inhibition by exposure to potent model TPO inhibitors. The DNA of larvae with putative TPO knockout was confirmed to have persistent mutations in the targeted regions of the TPO gene by Sanger sequencing, supporting the specificity of the apical effects. As demonstrated here with a well-characterized mechanism of thyroid toxicity, CRISPR/Cas9-mediated gene editing will support the linkages between a putative molecular target, downstream biochemical effects and adverse organismal outcomes, facilitating the advancement of predictive approaches to chemical safety evaluation.

**TP107 Pharmacokinetics of Anticoagulant Rodenticides in Largemouth bass and Black triggerfish**

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Anticoagulant rodenticides (ARs) have been used for decades with limited knowledge of acute and chronic effects on non-target species, especially fish species. Our previous studies have demonstrated a high concentration of ARs was needed to elicit mortality in fish we have tested. Little to no information on the ADME (absorption, distribution, metabolism, and elimination) of ARs in fish exists. Using sub-lethal intraperitoneal injection (IP) doses, we monitored ARs in various tissues over time to determine the pharmacokinetics of ARs. Our species of interest, largemouth bass (LMB, *Micropterus salmoides*) and black triggerfish (BT, *Melichthys niger*), were dosed with a sublethal concentration of a designated AR, diphacinone (DPN) and/or brodifacoum (BROD). Liver, muscle and plasma were analyzed for AR concentrations over a 120 hour period. Blood clotting assays were also conducted to determine changes over time due to AR exposure. In LMB, the greatest concentrations were observed in the liver (13.03 µg/g +/- 8.52) and plasma (12.36 µg/g +/- 5.68) at 48 hours post-exposure. Clotting times increased following DPN exposure, peaking at 96 hours (average 22 seconds), then decreasing at 120 hours (average 7.75 seconds) in LMB. Exposure to DPN in BT or BROD in LMB also demonstrated an increase (up to 72 hours) and subsequent decrease (96-120 hours) in clotting times. Estimation of clearance times of ARs in fish will aid efforts to determine the risks residues may pose due to secondary exposure.

**TP108 A combined PBTK and qAOP-modeling approach to assess the impact of DLC-induced embryotoxicity on recruitment failure in European eels**

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The panmictic stock of the European eel (*Anguilla anguilla*) has seen a dramatic decline over the past several decades, and declines in recruitment as a result of maternally transferred contaminants has been proposed as one of several potential causes. In particular, dioxin-like chemicals (DLCs) have been identified as a class of chemicals of great concern for both European and American eels (*Anguilla rostrata*). DLCs bioaccumulate, are highly embryotoxic in many species of fish, and maternally transferred in artificially matured eels. However, to date researchers have been unable to locate reproducing adult eels or developing embryos in their natural spawning grounds in the Sargasso Sea. As a result, accurate embryotoxicity data to identify the potential causative chemicals are unavailable. Therefore, this study aimed to (a) parameterize a physiologically-based toxicokinetic (PBTK) model for European eels to account for the impact of changes in physiology that result from sexual maturation and migration on toxicokinetics, and (b) to couple this model with a quantitative adverse outcome pathway (qAOP) for activation of the aryl hydrocarbon receptor 2 (AHR2) of fishes to predict early life stage mortality of eels as a result of exposure to maternally transferred DLCs. The PBTK model was used to kinetically predict the redistribution of DLCs within the body of female eels during migration, and ultimately the concentration in gonads and eggs. A simple qAOP was described previously linking activation of species-specific AHR2 in an in vitro luciferase reporter gene assay using transfected COS-7 cells with embryo lethality across nine species of fishes exposed to DLCs. To this end, AHR2 was cloned from European eel and used to predict eel-specific relative potencies of five DLCs representing congeners measured at among the greatest concentrations in gonads of eels. Using this data, mortality of early life stages of eels was estimated based on the internal concentrations predicted by the PBTK model. Our integrated PBTK model and qAOP approach will ultimately shed light on the question whether early life stage mortality induced by exposure to DLCs has the potential to significantly contribute to the observed decline in recruitment of eels.

## Identifying Unknown Causes of Toxicity: Proven and Innovative Methods

**TP109 Relevance of the formation of reactive electrophiles during oxidative water treatment**

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Oxidative treatment technologies that utilize oxidants such as hydroxyl radicals, ozone, sulfate radicals and chlorine are increasingly used in various water treatment applications, including wastewater, water reuse, drinking water and remediation of groundwater at contaminated sites. While these technologies are very effective in removing a large spectrum of organic contaminants, knowledge about the fate of these chemicals, in particular the formation of transformation products is still widely lacking. The elucidation of transformation products is further complicated by the presence a large number of natural organic compounds as these also can be precursors of toxic transformation products formed during chemical oxidation. As a result, treated waters typically contain a complex mixture of organic compounds. While modern analytical tools, in particular

high-resolution mass spectrometry, enable the detection of thousands of compounds in a single water sample, they do not provide any information about their toxicological relevance. On the contrary, in vitro or in vivo assays, which are frequently used for a toxicity assessment, provide information about the overall toxicity of a mixture while the identities of responsible toxicants remain unknown. To address this, we investigated the formation of electrophilic transformation products during oxidative water treatment by analyzing their reactions with biomolecules, in particular amino acids, peptides and proteins. Using this approach, we were able to identify a,b-unsaturated dicarbonyls such as 2-butene-1,4-dial as previously unknown transformation products that are formed during oxidation of phenols by hydroxyl radicals or chlorine. The formation of 2-butene-1,4-dial is of particular concern due to its known high mutagenicity. The used methodology thus offers a powerful approach to identify toxic transformation products that are formed during oxidative water treatment.

**TP110 Applying High Resolution Mass Spectrometry to Identify Toxic Chemicals in Wastewater Effluents**

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Intermittent but substantial effluent toxicity to the freshwater invertebrate *Ceriodaphnia dubia* has been experienced by a large POTW (>150 mgd) with a substantial industrial user input, with a gradient of effects typified by reversible paralysis, reproductive impairment, and mortality. Results of conventional toxicity identification evaluation (TIE) testing with *C. dubia* suggest the contaminant(s) is organic, relatively polar in nature, and possibly charged, where the unifying commonality across all effective TIE treatments is the high surface area of potentially sorptive materials. The most recent effluent toxicity episode, where only the first two of six total 24-hour effluent composite samples were chronically toxic to C. dubia, presented a unique opportunity to apply high resolution mass spectroscopy to the identification of possible contaminants. In this study, both liquid and gas chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS and GC-QTOF-MS) were used to quantify target compounds, screen for >5,000 suspect compounds in high resolution MS databases, and to search for unidentified ions that were correlated with the degree of toxicity observed across the sample time series. LC-QTOF-MS data were acquired in All-Ions fragmentation mode in both positive and negative electrospray ionization (ESI). LC suspects were identified using two accurate mass libraries containing pesticides, pharmaceuticals and other environmental contaminants. GC-QTOF-MS data were acquired in electron ionization (EI) mode and negative chemical ionization mode (NCI). The overall analytical workflow and data analysis approach followed methods similar to those developed by Moschet et al. (Environ. Sci. Technol., 51:1553, 2017). A total of 29 suspect compounds were detected by LC-QTOF-MS and qualified by screening against the databases and six target compounds were detected by GC-NCI. None of these compounds had a statistically significant ($p< 0.05$) linear correlation with concentration or area counts and the observed mortality or reproductive effects of that sample, suggesting that none are the primary agents of toxicity. Nontarget analysis of the LC data isolated more than 1700 compounds per sample in ESI- and over 2400 compounds per sample in ESI+. A total of 232 of these compounds were uniquely present in the samples displaying toxic outcomes. Approaches to further identify these compounds and connect them with the toxicity observed will be discussed.

**TP111 Using High Resolution Mass Spectrometry to Identify Contaminants and Sources Linked to Urban Stormwater Mortality Syndrome in Coho Salmon**

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Urban stormwater is a major water quality threat to ecological health, causing a range of adverse, but mostly sublethal, effects in aquatic species. In western North America, untreated urban runoff induces pervasive acute mortality in adult coho salmon (*Oncorhynchus kisutch*). To better understand the relationship of this phenomenon to water quality, we applied non-target and suspect screening analyses using high resolution mass spectrometry (HRMS) to detect non-target and identified chemical features that co-occurred in sampled waters that were lethal to coho salmon in the lab and the field. Hierarchical cluster analyses were used to evaluate potential causal sources; these analyses indicated that tire wear particle (TWP) leachates were most similar to the waters that induced coho mortality, relative to other vehicular sources (e.g., motor oil, antifreeze). Among prominent detections in the mortality signature, we identified several groups of pervasive contaminants in road runoff, including polyethylene glycols (PEGs), octylphenol ethoxylates (OPEOs), polypropylene glycols (PPGs), and two groups of N-containing compounds derived from TWP. In particular, a family of (methoxymethyl) melamine compounds, previously unreported in United States surface waters, were detected in highway runoff at concentrations up to ~9 μg/L and in urban creeks at concentrations up to ~300 ng/L. Based on the prioritization of TWP as an important source in urban runoff and as relevant to the urban coho mortality syndrome, occurrence of the identified compounds and the coho mortality signature were evaluated in Puget Sound surface waters and during controlled exposures of coho salmon.

**TP112 Optimization of Sediment Toxicity Identification Evaluation Study Designs to Account for Spatial and Temporal Variability**

*D.J. Greenstein, A.N. Parks, S.M. Bay, Southern California Coastal Water Research Project / Toxicology*

Toxicity tests are an important aspect of sediment quality assessments, but knowledge of the cause of toxicity is needed to determine effective management actions. Toxicity Identification Evaluation (TIE) methods were developed to meet this need. While TIE methods manuals have detailed descriptions on performing the treatments, there is little guidance on study design, including the number of TIE samples needed to confidently make an assessment. Additionally, the level of variability associated with performing TIEs and how to account for it is not addressed. We conducted a literature survey and found that in 19 of 45 published investigations only one TIE sample was tested to determine the cause of toxicity. The goal of our study was to collect data on both the spatial and temporal variability associated with sediment TIEs using the amphipod *Eohaustorius estuarius* 10-day survival test and then use that information to make recommendations for designing future TIE studies. Ten stations were sampled in two stages at Consolidated Slip in Los Angeles Harbor, California, USA, with samples collected two months apart. In Stage I, extensive TIEs were conducted on whole sediment and pore water from three of the most toxic stations. In the Stage II, more focused TIEs were conducted on whole sediment from all ten stations. Chemical analysis for metals and a suite of organic contaminants was performed on all samples where TIEs were conducted. Using a weight of evidence approach, including TIE and chemistry analysis, it was determined that pyrethroid pesticides were the likely cause of observed toxicity at Consolidated Slip, with a lesser contribution from PAHs. Results of the 13 individual TIEs fell into three broad categories: seven TIEs where treatments for organic chemicals and specifically pyrethroids were effective; three where the treatment for pyrethroids was not effective but the treatment for organic contaminants was effective; and three where the treatment for pyrethroids was effective, but the treatment for organic contaminants was not effective. This variability was used in calculations which determined that at least three TIEs were necessary to make a confident assessment of the cause of toxicity. The results indicated that there was not substantial temporal variability in the TIE outcomes. Other recommendations are made regarding effective TIE study design.

**TP113 The Unsuccessful Toxicity Identification Evaluation**

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Whole Effluent Toxicity Testing (WET) can drive regulatory requirements to carry out Toxicity Identification Evaluation (TIE) testing or require a Toxicity Reduction Evaluation (TRE) on effluents not meeting permitted limits. The WET program has been successful in removing and reducing effluent toxicity through the NPDES permitting process, however, an increased number of effluents have been minimally and/or intermittently toxic. This scenario has created issues with the completion of a successful TRE program. The consequences of unidentified toxicity are uncertain treatment measures and costly and possibly endless testing to regain compliance. This presentations outlines and discusses issues and strategies that have been utilized during TRE evaluations, where difficulty identifying the causative toxicants has been encountered. The topics addressed include: non-persistent toxicity; minimal toxicity samples; and ephemeral toxicity.

**TP114 Amending the Standard Phase I Toxicity Identification Evaluation: Four Useful Additions**

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Wastewater treatment plant facilities that discharge unacceptable levels of toxicity must perform a Toxicity Reduction Evaluation (TRE) to identify and control the causes of toxicity. One common component of a TRE is the application of USEPA's Toxicity Identification Evaluation (TIE) procedures to identify and confirm the causes of toxicity. However, in many instances the application of the standard procedures (pH adjustment, aeration, filtration, C18 extraction, EDTA addition, sodium thiosulfate addtion, activated carbon and graduated pH) yields insufficient evidence to identify the causative toxicants associated with the wastewater. The utilization of non-standard TIE methodologies has become increasingly important as the increased efficacy of wastewater treatment has resulted in the removal of the majority of classical toxicants in industrial wastewaters, which are most commonly removed through standard TIE methodologies. This presentation provides case study evaluations of four non-standard TIE treatments: 1) Sulfamic acid (nitrite); 2) Kaolinite clay (polymers and surfactants); 3) Activated Alumina (Flouride); and 4) Ferric Chloride (Chlorite).

**TP115 Toxicity of Lead and Copper in Elevated Dissolved Organic Carbon Effluent to *Ceriodaphnia dubia***

*A.M. Briden, N. Lynch, S. Vasquez, B.C. Jorgenson, Pacific EcoRisk*

In this study we evaluate the impact of elevated dissolved organic carbon (DOC) on the acute toxicity of Pb and Cu in effluent to *Ceriodaphnia dubia*. Acute 48-hour testing with *C. dubia* was performed following standard USEPA methods. Metals concentrations were analytically verified in the test solutions to allow for determination of dissolved Pb and Cu $EC_{50}$ values. The Cu and Pb Biotic Ligand Models (BLM) were both used to calculate predicted Cu and Pb effect thresholds for comparison to the measured values generated in this study. Although the effluent DOC concentrations exceeded the upper calibration limit of the BLM, the measured and predicted effect thresholds were comparable.

**TP116 Application of Phase I and Phase II TIE Techniques in the Identification of Nickel and Vanadium Toxicity in an Industrial Effluent**

*A.M. Briden, S.L. Clark, B.C. Jorgenson, Pacific EcoRisk*

The cause of unknown chronic toxicity to *Ceriodaphnia dubia* in a limestone quarry discharge was investigated and elucidated through the execution of Phase I and Phase II toxicity identification evaluations (TIE). Initial Phase I TIE testing identified chronic toxicity associated with cationic and anionic constituents, with analytical chemistry identifying nickel (Ni) and vanadium (V) as potential causes of chronic toxicity that warranted further investigation. Phase II TIE testing employing Ni-selective sorbents, a Ni and V spiking study utilizing buffered and unbuffered laboratory water, and a synthetic effluent study all confirmed the significant contribution of Ni and V to the observed chronic toxicity. Field-based pilot scale treatment studies confirmed removal of toxicity associated with substantial reductions in Ni and V concentrations.

**TP117 Variability in Sediment Toxicity and TIE Response: An Underappreciated Contributor to Unknown Toxicity in California Bays**

*S.M. Bay, A.N. Parks, D.J. Greenstein, Southern California Coastal Water Research Project / Toxicology*

Sediment toxicity testing with amphipods and TIE characterization treatments remain the most direct and effective methods for determining the cause of sediment quality impairment in marine sediments and detecting unmeasured toxicants. However, TIE characterization treatments targeted towards nonpolar organics, metals, and ammonia occasionally fail to indicate the chemical class likely associated with the toxicity, suggesting that unmeasured contaminants or non-contaminant stressors (e.g., sediment particle size) may be the toxicant. Inability to identify the likely cause of sediment toxicity is an impediment to developing effective management and regulatory actions to improve sediment quality. Variability in sediment toxicity test responsiveness and TIE treatment performance have a strong influence on TIE results, yet TIE study designs may not allow such effects to be recognized. Recent TIE case studies from two locations in Southern California, Marina del Rey Harbor and Los Angeles Harbor, are examined to illustrate the impact of test variability on TIE interpretation. In Marina del Rey Harbor, TIE characterization treatments were unsuccessful in determining the cause of moderate levels of sediment toxicity. Furthermore, sediment toxicity disappeared at the stations over a six-month period, even though there were no changes in sediment concentrations of priority pollutants and current use pesticides. In Los Angeles Harbor, multiple stations with high sediment toxicity were characterized using TIE treatments that included addition of charcoal, cation exchange resin, zeolite, and PBO. Sediment chemistry and spatial analysis indicated that all test sites had similar contamination profiles and that pyrethroid pesticides were causing toxicity at each site. However, effectiveness of the charcoal and PBO treatments varied among sites, leading to potentially different conclusions regarding the cause of toxicity at individual stations. Recommendations for improvements to TIE experimental designs and quality assurance practices, as well as new methods with greater sensitivity and specificity, are suggested that will help maintain the value of TIEs for sediment quality assessment.

**TP118 Effects of glyphosate on the cell population growth of the green algae *Selenastrum capricornutum*, and removal of toxicity with TIE treatments**

*A. Cibor, Enthalpy Analytical / Aquatic Toxicology; K.M. Payne, P. Arth, Enthalpy Analytical / Nautilus Environmental / Aquatic Toxicology*

Cell population growth of the freshwater aquatic green algae *Selenastrum capricornutum*, a common monitoring species for toxicity testing, can be affected by the practice of spraying herbicides near potential points of sample collection. This can include treatment settling ponds, WWTP effluent discharge/sampling points, urban storm water outfalls, and natural receiving waters. Many of these water bodies are subject to NPDES permit monitoring, and introduction of these herbicides (especially outside of a wastewater treatment process that would remove them) may have an impact on compliance results reported to regulatory agencies. Often these herbicides can be introduced by processes that are outside of the control of responsible parties. This presentation explores three primary objectives: 1) establishing baseline toxicity data for glyphosate effect levels, 2) assessing if those levels could potentially be observed in an effluent or receiving water sample, and 3) finding effective toxicity identification evaluation (TIE) treatments for glyphosate-related toxicity. Spiking studies using glyphosate, a common ingredient used in many weed killers found in most home improvement or hardware stores, were conducted to determine effect concentrations in green algae. Once effect concentrations were established, an evaluation of TIE treatments was conducted on the glyphosate-spiked samples in order to identify a single treatment or combination of treatments that would effectively remove the toxicity. 96-hour growth tests were conducted according to EPA 2002 freshwater chronic test methods (EPA-821-R-02-013). Additionally, an evaluation was performed to determine the persistence of the observed toxic effect in an effort to identify whether a TIE could be effectively performed within a reasonable timeframe after initial toxicity was detected. Finally, studies were conducted to establish if, when applied according to manufacturer specifications, glyphosate could be measured in quantities high enough to elicit a toxic response in the green algae test.

## Interpreting Biological Effects of Metals and Their Mixtures in the Aquatic and Terrestrial Environments

### TP119 Aluminum and Acetylcholinesterase in Honey Bees: Understanding Interactions Using Behavioral and Biochemical Data

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Aluminum is increasingly globally bioavailable with acidification from warming and poor mining practices. This bioavailability has been shown to increase uptake by flora that can then be dispersed through products such as fruit, pollen, and nectar. Concentrations of aluminum in fruit and pollen in North America have been reported between 0.05 and 670mg/L. This is particularly concerning for pollinators that ingest pollen and nectar. Honey bees represent a globally present species experiencing decline in Europe and North America. Region specific decline may be a result of differential toxicity of toxicant ingestion by subspecies. Previous literature suggests that aluminum binds to acetylcholinesterase and limits the protein's activity. We have previously shown that low concentrations of ingested aluminum may temporarily increase activity and decreases circadian rhythmicity and lifespan, but that these effects are partially dependent on subspecies. These data also suggest a possible hormetic effect of aluminum in honey bees that warrants further study at intermediate doses. The behavioral and mortality results corroborate the cholinergic hypothesis however this analysis has not been previously conducted in honey bees. We conducted a cholinergic enzyme analysis in the form of a modified Ellman assay on *Apis mellifera mellifera* heads. We exposed live bees to various concentrations of aluminum in sucrose solution for up to 48 hours before analysis. We expect that exposure to aluminum will decrease enzyme activity which will confirm the cholinergic aluminum hypothesis in honey bees.

### TP120 Assessing effects of copper and zinc mixtures on larval development rates of the Mediterranean mussel (*Mytilus galloprovincialis*)

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Commercial and recreational boat hull anti-fouling paints often contain a variety of different metals which can have detrimental effects on marine organisms in harbors and marinas. While copper is still a common and effective ingredient in most hull anti-fouling paints, concerns about copper toxicity have led to an increase in low-copper or non-copper paints that may use zinc as an alternative. Whole Effluent Toxicity (WET) testing has been used as part of the evaluation framework to determine water quality criteria for contaminants of concern such as metals. However, because marine organisms are likely to be exposed to multiple metals, particularly where anti-fouling paint in present, there has been increased interest in understanding the combination effects of metals. Exposure to combinations of metals can potentially yield different effects than those observed when an organism is exposed to a single metal. Mixtures can lead to a variety of responses such as additive, synergistic, or deleterious effects, and understanding these interactions at the species-specific level can provide valuable information for decision makers. While there have been many previous studies examining the effects of copper on mussel development, fewer have focused on zinc and copper mixtures. Bivalves are of particular interest as they are both a target organism for anti-fouling paints as well as a common monitoring species used to evaluate ambient water quality in harbors and marinas. This study investigates the combined effects of copper and zinc on the embryo-larval development of the Mediterranean mussel (*Mytilus galloprovincialis*) using published EPA methods. Environmentally relevant levels were evaluated, with many of them representing measured concentrations in previously tested ambient samples. Observed responses for the various ratios of combined copper and zinc exposures were evaluated against effect levels for the individual metals. This study highlights the importance of considering effects of metals mixtures when determining site-specific criteria for individual water bodies.

### TP121 Comparison of the ecotoxicological effects on aquatic organisms of the last two spills related to iron mining in Brazil

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In the last 3 years, 2 spills related to iron mining occurred in Brazil. In November 2015, with the rupture of the Fundão dam in Mariana/MG, about 50 million m³ of tailing traveled 650 km in the Doce River watershed towards the ocean. In March 2018, with a pipeline spill, around 500 tons of iron ore reached Santo Antônio do Grama stream (MG), and the plume traveled 21 km until the confluence with Casca river. Among possible environmental alterations in such events, the chemical contamination by the main constituents of the ore or tailing, iron and manganese (APLYSIA, 2015; GOLDER, 2016) and the impact associated of the input of solids in the water are highlighted. Indicators based on effetcs were used in both spills to evaluate the possible impact to the aquatic organisms. Standardized assays were conducted, according to technical regulations, with organisms of 3 different trophic levels: the fish *Danio rerio*, the microcrustaceans *Daphnia silimis* and *Ceriodaphnia dubia*, and the bacteria *Vibrio fisheri*. In the first event, 1048 results were gathered, collected in 45 sites from November 2015 to January 2016; in the second event, 482 results were obtained in 17 sites from March to April 2018. In the 2015 spill, 98.75% of the acute assays indicated an absence of toxicity, but 77.89% of chronic assays (*C. dubia*) indicated presence of toxicity. The samples with toxicity coincided with the moment of passage of the tailings plume, indicating that such impact may be associated to the physical effects of suspended solids (MELLO et al, 2017). In the 2018 event, 100% acute effects absence and 48.48% chronic ecotoxicity presence for *C. dubia* were observed. It was verified a spatial relation between the toxic results and the sites under greater influence of the spill, mainly due to removal of the material. The relation with the suspended particles was observed during moments of intense precipitation, when toxic effects were registered including sites with no influence of the spill (MELLO et al, 2018). *C. dubia*, a filter organism, is affected by concentrations of suspended particles due to obstruction of their feeding structures, which affects the growth rate and promote stress to the organism (HYNES, 1970). In the end, despite the events of distinct dimensions, the same response patterns were observed: absence of acute ecotoxicity and presence of chronic ecotoxicity for *C. dubia*, mainly related to the concentrations of suspended solids on the water.

**TP122 Metal Mixture Toxicity and Accumulation in Lettuce Seedlings**

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Many toxicants are found in the environment as mixtures but because toxicants are routinely tested individually, mixture effects are poorly understood. Understanding the biological effects of realistic exposures, like mixtures, is essential to managing environmental contamination. In this study we explored how the presence of one metal affects the bioavailability and toxicity of a second metal when present in tandem. In addition to toxicity testing, we measured accumulated concentrations of metals in exposed lettuce seedlings. This data could be used within the tissue-residue approach (TRA) for toxicity assessment, which uses internal tissue concentrations as a dose metric rather than ambient aqueous concentrations. This approach removes exposure variability and allows for more accurate prediction of toxicity under expected environmental conditions. For this experiment we conducted acute toxicity tests with lettuce seedlings exposed to binary metal mixtures and used growth inhibition as the effect endpoint. The first step was to determine the tissue accumulation and toxicity of single metals (Al, Cu, Cd, Fe, Zn). Next, we exposed seedlings to all combinations of binary mixtures and measured growth inhibition and bioaccumulation of metals. We used toxic units and the Isobole approach to classify the mixture effects as additive, synergistic or antagonistic. The toxicity results using nominal concentrations have shown antagonistic interactions for all mixtures tested, except Fe and Cu. The tissue concentration data collection is on-going and results will be presented. Primarily applied to organic mixtures, experimental studies that utilize the TRA approach to describe metal mixture toxicity are rare. This work improves our understanding of mixture effects and bioavailability and is critical to predicting toxicity of metals in the environment under expected environmental conditions.

**TP123 The Trouble with Toxic Units**

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Risk assessments conducted on chemical mixtures under the assumption of additivity have the potential to underestimate or overestimate risk depending on the slopes of concentration-response relationships for each chemical. Chemical mixtures occur ubiquitously in the environment and are a growing concern amongst various governmental and private sector industries. Due to the complexity of many of the naturally occurring mixtures, mixture toxicity is generally poorly characterized. One method of standardizing multi-chemical toxicity is known as the toxic unit (TU) approach, where a TU is defined as the ratio of the concentration of a given chemical in a mixture to the concentration that would elicit toxicity in 50% of a population (EC50). Because the toxicity of different chemicals can vary widely on a mass basis, a TU can be a powerful tool to standardize the metric of toxicity between multiple chemicals, and in the concentration-addition (CA) model, the sum of TUs from multiple chemicals can be used to generate a mixture toxicity prediction. In many systems, however, chemical concentrations occur well below the EC50, and because calculation of a TU does not consider the slope of the concentration-response relationship, it becomes nearly impossible to determine the effect level from TUs. For instance, a TU = 0.5 for a chemical with a shallow dose-response curve will exhibit higher toxicity than a chemical with a steep dose-response relationship. For this reason, the CA model is primarily recommended for chemicals with similar concentration-response slopes. The goal of this study is to quantify how differences in individual-chemical concentration-response curves affect predicted mixture TUs. For the analysis, mixture TU predictions were generated from binary combinations of simulated dose-response curves with (1) both having shallow slopes, (2) both having steep slopes, and (3) one steep slope and one shallow slope. Next, mixtures containing more than two chemicals were evaluated in relation to the binary results to determine if the addition of more chemicals with either the same or different slopes compounded or mitigated the differences in the predicted mixture toxicity. Finally, these results were compared to the independent-action model predictions to draw conclusions with respect to the applicability of the different additivity models.

## Micro-organisms and Biopesticides in Crop Production: Use and Environmental Safety Considerations

**TP124 Current environmental regulations might not protect against non-targeted effects of microbial pesticides: The case of Dipel and Daphnia**

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Currently, there is a trend toward an increasing use of biopesticides assumed to be environmentally friendly, such as *Bacillus thuringiensis* (Bt). Nonetheless, the introduction of microorganisms as prospective contaminants might add environmental risks and uncertainty as these organisms likely respond to environmental cues different from inanimate matter. For instance, studies of the Bt toxicity to nontarget organisms have reported low effects at high exposure levels, which is interpreted as indicating negligible risk to nontarget organisms. To test whether the Bt-based biopesticide DiPel ES fulfilled the core ecotoxicological assumption of monotonicity (e.g. toxicity increasing with increased contaminant exposure), we investigated the response of the nontarget organism *Daphnia magna* to waterborne DiPel ES, a globally used Bt formulation. Neonates and adults were exposed for 48 h to more than 25 concentrations covering more than 4 orders of magnitude. Immobilization and mortality were monitored in more than 1000 organisms (567 neonates and 578 adults), representing the largest single study of Bt nontarget toxicity. Whole body biomarkers (body weight, protein, chitobiase, catalase, xenobiotic metabolism, and acetylcholinesterase) were measured in the adults. The high concentrations of this biopesticide did not cause significant effects, however low doses did elicit high toxicity. In other words, the immobilization and mortality of the neonates were affected in a nonmonotonic and inverted U-shaped pattern with $EC50_s$ that were ~$10^5$-fold lower than those reported by the manufacturer. The immobilization of adults demonstrated a similar pattern. The biomarker results revealed multiphasic dose-response curves, which suggested toxicity mechanisms that affected various physiological pathways. The main particle size in exposure media was in the size range of bacterial spores and crystal toxins. However, the chemical heterogeneity was nonmonotonic, with a change in the phase at the maximum of toxicity (~5 $\mu L\ L^{-1}$), which might explain the observed nonmonotonic effects. These results demonstrate the vulnerability of a nontarget organism to a biopesticide that is considered to be safe, while challenging the applicability of the central ecotoxicological assumption of monotonicity. In this sense, microorganisms may differ from traditional agrichemical contaminants, thus requiring new ecotoxicological frameworks for proper regulation.

**TP125 Environmental Safety Assessment for Agricultural Uses of Lipochitoligosaccharides as Plant Growth Regulators**

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Lipochitooligosaccharides (LCOs) are molecular signaling molecules involved in plant-microbe symbioses, including rhizobial nodulation by bacteria and arbuscular mycorrhization by fungi. The bacteria and fungi involved in these natural processes produce a wide variety of LCO molecules, but they act via the same receptor-mediated processes in plants. LCO signaling progresses via the DMI (doesn't make infections) pathway and can result in nodulation, root branching, and mycorrhization. Biological agricultural products developed in collaboration between Monsanto and Novozymes capitalize on the ability of LCO molecules to enhance plant health. To support registration of products containing LCO SP104 and LCO MOR116 in the United States, the environmental safety of these two LCO molecules was evaluated through nontarget organism studies performed according to US Environmental Protection Agency guidelines. Taxa evaluated included mammals, birds, fish, aquatic invertebrates, insects, and plants. In acute toxicity tests, LCO SP104 and LCO MOR116 were found to be practically nontoxic to all tested taxa. LCO SP104 did not have adverse effects on plants, even at concentrations above the maximum label rate. Considering the low use rates of agricultural products containing LCO SP104 and LCO MOR116, maximum predicted exposures to LCOs are orders of magnitude less than those evaluated in the nontarget organism studies. Additionally, animals and plants have a history of safe exposure to LCOs originating from the rhizosphere, and LCOs degrade into molecules that are essentially nontoxic, such as chitin and vaccenic acid. For these reasons, the potential for risk to nontarget organisms from agricultural uses of LCO molecules is negligible.

## Incorporating Effect-Based Molecular Assays in Environmental Monitoring and Risk Assessment

**TP126 Evaluating the Effectiveness of Advanced Oxidation Processes at Reducing AhR, ER, and p53 activity via In vitro Cell Bioassays**

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Persistence of emerging compounds in wastewater effluent is a concern that has received increasing research interest over the past decade particularly in water scarce regions such as California. As sources of recycled water have been increasing, it is imperative to develop methods that can evaluate the safety of recycled water and the treatment processes used to enhance water supplies. To analyze the effectiveness of water treatments, in vitro cell bioassays were performed using water samples taken from the different stages and types of treatment. Secondary effluents were chlorinated, chlorinated and brominated (C+B), or nonhalogenated (control). The treatment train also utilized different advanced oxidation processes (AOPs), including microfiltration and reverse osmosis. Nonhalogenated samples were found to elicit significant receptor activity in both the p53 and ER assays. Chlorination reduced estrogenicity in general, with statistically significant reductions in receptor activity for the microfiltration treatment process. In addition, C+B was found to be effective at reducing p53 activity in the UVA/OP and reverse osmosis samples. While not below detection limits, this AOP also proved to be more effective at reducing p53 activity in secondary effluent and untreated Santa Ana River water. AhR activity for all samples was found to be insignificant. These results are important for evaluating the potential impacts of recycled water.

**TP127 Pollutant-Induced Changes in Ca2+ Channels Alter DREAM-Mediated Gene Transcription**

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Several environmental pollutants, including polychlorinated biphenyl (PCB) congeners and triclosan, are capable of causing $Ca^{2+}$ signal disruption (CSD) by altering the activity of the ryanodine receptor (RyR) or the L-type voltage gated $Ca^{2+}$ channel (CaV1). These two channels are important to countless physiological processes, but the extent to which CSD through these channels contributes to altered cellular pathways is currently unclear. We investigated whether CSD, caused by cellular exposure to PCBs and triclosan, alters gene transcription as regulated by the $Ca^{2+}$-sensitive transcriptional repressor DREAM (downstream regulator element antagonistic modulator). When intracellular $Ca^{2+}$ concentrations are decreased, DREAM remains bound to DNA and represses transcription, and at high intracellular $Ca^{2+}$ concentrations, DREAM is released from DNA and activates transcription, representing a direct connection between CSD and transcription. We utilized the GT1-7 hypothalamic and the TαT1 thyrotrophic cell lines to measure whether CSD alters transcription of gonadotropin releasing hormone (GnRH), thyroid stimulating hormone (TSH), neuronal PAS domain protein 4 (NPAS4) and brain-derived neurotrophic factor (BDNF). Cells were exposed to varying concentrations of each pollutant for multiple time periods and GnRH, TSH, NPAS4, and BDNF levels assessed using qPCR. GT1-7 cells exposed to the potent RyR activator PCB 95 did not lead to changes in GnRH mRNA expression consistent with low basal RyR gene expression. Exposure of GT1-7 cells to triclosan, a CaV1 inhibitor, significantly decreased GnRH transcription in a concentration and time dependent manner. Exposure of TαT1 cells to PCB95. a RyR activator, showed no significant changes in TSH, and beginning analysis of NPAS4 and BDNF transcription levels are trending upward. Small interfering RNA (siRNA) knockdown followed by DREAM rescue experiments are currently being conducted. DREAM is important to proper functionality of the digestive system, central nervous system, and skeletal and cardiac muscle, and it has been tied to pain reception, learning and memory and thyroid-gland health. This work will help address whether CSD is contributing to such alterations by altering DREAM-mediated transcription.

**TP128 Screening of neurodevelopmental toxicity of nanomaterials : Embryonic stem cell, neural stem cell and zebrafish study**

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Neurotoxicity and developmental toxicity of nanomaterials have been previously reported. However, neurodevelopmental toxicity of nanomaterials has still been poorly understood. In this study, to elucidate neurodevelopmental toxicity due to exposure of nanomaterials, we investigated developmental toxicity of nanomaterials using zebrafish embryos (in vivo) and embryonic stem cells (in vitro) and neurodevelopmental toxicity of nanomaterials using neural stem cells (in vitro). Various nanomaterials, such as, silver nanoparticles, $SiO_2$, MWCNT and graphenes were screened using these in vitro and in vivo models. The preliminary results suggests possible involvement of nanomaterials on neurodevelopmental processes. Our result also suggests multi-model approach could complement the limitations of a stand-alone model organism and thus more accurately identify hazard of nanomaterials. Acknowledgement : This research was supported by Nano Material Technology Development Program through the National Research Foundation of Korea(NRF) funded by Ministry of Science and ICT (2014M3A7B6020163).

**TP129 Contaminant body burdens and molecular responses associated with dietary exposure to mercury and POPs in the little skate (*Leucoraja erinacea*)**

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Elasmobranchs are globally threatened by environmental stressors due to their life histories as long-lived, slow-growing apex predators with slow maturation and low fecundity. Over-harvesting is recognized as a major threat, but given life-history considerations, elasmobranchs may be at additional risk from exposure to contaminants including mercury and persistent organic pollutants (POPs) such as PCBs and other halogenated organic compounds. Contaminant bioaccumulation and potentially negative consequences of their accumulation in elasmobranchs is understudied, with relatively little published work available on only a few species. In order to address this issue, we conducted a pilot study to examine contaminant bioaccumulation and cellular responses in juvenile little skates (*Leucoraja erinacea*) exposed to a diet rich in POPs. Two groups of little skates were exposed to a sushi-grade tuna diet high in methyl mercury and POPs or a reference diet of farmed salmon known to be low in POPs. After 20 days of dietary exposure, muscle and liver tissue were harvested from the skates. Mercury levels measured in liver and muscle tissue were several times higher in tuna-fed skates ($Hg_{liver}$=0.711 mg/g d.w., $Hg_{Muscle}$=0.707 mg/g d.w.) than salmon-fed skates ($Hg_{liver}$=0.251 mg/g d.w., $Hg_{Muscle}$=0.185 mg/g d.w.), and a significant correlation was observed between Hg levels in muscle and liver tissue on an individual basis ($R^2$= 0.838, p= 0.00141). We are in the process of analyzing diet and tissue samples for a suite of POPs including 41 PCB congeners and 19 organochlorine compounds. If similar correlations are observed with other POPs, this would support the utility of using non-destructively collected muscle biopsies to evaluate POP body burdens in wild-caught organisms. Additionally, we will be using quantitative PCR to evaluate differential RNA expression of a suite of transcripts known to be associated with POP exposure and effects to determine if important cellular systems may have been modified by the relatively natural exposure modeled in this experiment. Targeted transcripts include glutathione-*S*-transferase (GST), metallothionein (MT), heat shock protein 70 (HSP70), and CYP1A. This study will be one of the first to compare bioaccumulation and cellular response in elasmobranchs under controlled exposures and will support future work with additional wild caught species examining the impact of bioaccumulative chemical stressors on elasmobranch cellular systems.

**TP130 RNA-sequencing analyses to better understand how temperature or parasitic infection modulates cadmium toxicity in rainbow trout (*Oncorhynchus mykiss*)**

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The simultaneous presence of natural and anthropogenic stressors in aquatic ecosystems can challenge the identification of factors causing declines in fish populations. These stressors may include chemical mixture and the potential interactions with environmental factors, such as water temperature and parasitism. The stressor-stressor interactions may vary from antagonism to synergism resulting in environmental risks at the organismal or population level not predicted by exposure to individual stressor. This study aimed to evaluate the combined effects of cadmium (Cd) and elevated water temperature or parasite infection in juvenile rainbow trout (*O. mykiss*). Fish were exposed to diet borne Cd (6 µg Cd/g wet feed), individually and in combination with thermal (23°C) or parasitic stress, for 28 days. The parasite challenge consisted of a single exposure to glochidia (larvae) of the freshwater mussel *Strophitus undulatus* which encyst in fish tissues. Results indicated lower fish length, weight, and relative growth rate in fish exposed to the elevated water temperature condition. Body condition and hepato-somatic index of fish were, however, higher in this temperature treatment compared to other groups. Hepatic Cd concentrations were 10-fold higher in trout fed with the metal-contaminated diet; exposure to thermal stress or parasitism did not influence tissue Cd bioaccumulation. More than 700 genes were differentially transcribed in fish exposed to the individual heat stress treatment, however, Cd exposure nor parasite infection did not affect the number of differentially transcribed genes, compare to controls. The highest number of differentially transcribed genes (969 genes) was observed in trout exposed to combined Cd and high temperature stressors. Only 40 genes were differentially transcribed when fish were exposed to Cd and glochidia. These results suggest that dietary Cd may exacerbate the water temperature stress and to lesser extend parasitic infection stress on trout transcriptomic responses. Genes involved in stress response, protein folding, angiogenesis, apoptosis and immune system were upregulated in fish exposed to Cd and heat, and genes involved in calcium, glucose and cholecalciferol metabolisms were downregulated. Biomarker analyses will be conducted to complement transcription analyses. This study may help identify stressor-response relationships in freshwater fish and potentially benefit to the environmental risk assessment and management.

**TP131 Marine sentinel species biomonitoring with gene expression: Comparing contaminant burdens and gene expression among bird breeding colonies**

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Persistent contaminants can bioaccumulate in marine birds, potentially causing injury or even mortality at higher levels of exposure. Additionally, the potential for catastrophic environmental disasters, for example, petroleum spills due to vessel traffic and bitumen transport in the eastern North Pacific Ocean, underscores the need to collect baseline tissue-contaminant data and improve wildlife injury assessment capabilities. Using avian sentinel species for continental shelf and nearshore habitats, the rhinoceros auklet and double-crested cormorant, respectively, we aim to demonstrate the utility of a gene expression-based approach for linking contaminant burdens to transcript-level perturbations among breeding colonies, and potentially individuals. Ongoing research using eggs collected at breeding colonies quantified a range of persistent organic pollutants, polycyclic aromatic hydrocarbons, and mercury in developing embryos. We also measured the relative expression of ≥27 genes involved in a variety of pathways such as xenobiotic and lipid metabolism, immune function, and oxidative stress response in embryonic liver tissue using species-specific PCR arrays. Thus, this comparison of contaminant concentrations in sentinel species among colonies coupled with gene expression should improve our understanding of contaminant effects in sentinel marine wildlife, while also evaluating the utility of individual genes or suites of genes as a biomarker of contaminant burdens in wild avian species.

**TP132 Characterizing changes in molecular toxicity pathways to predict adverse outcomes of fluoxetine in adult fathead minnows**

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Pharmaceuticals and Personal Care Products have become a major threat to aquatic environments due to their toxicity to non-target organisms and constant discharge into surface water systems. Fluoxetine (FLX) is the most common selective serotonin reuptake inhibitor pharmaceutical used for the treatment of psychological disorders such as depression. Increased prescription use of FLX by human populations has lead to its pseudo-persistence in effluent-receiving waterways. However, there is currently limited information on chronic, low-level exposure effects of FLX in fish. In particular, little is known about its specific mechanism of action and the associated toxicity pathway(s) in fish. This information is important for predicting apical responses to FLX. Therefore, the main goal of this study is to identify and validate key molecular toxicity pathways that are predictive of apical endpoints induced by exposure to FLX using a model species common to North American freshwater systems, namely the fathead minnows (*Pimephales promelas*). This will be accomplished by exposing adult fathead minnows to a range of three concentrations of FLX in a flow-through system for an exposure period of 21 days. After 96 h a subset of samples will be collected to characterize molecular toxicity pathways using whole transcriptome, whole proteome and targeted metabolome analyses. In addition, over the 21-day course of the experiment individuals will be assessed for behavioural changes, morphometrics, fecundity, and altered secondary sex characteristics, and these physiological outcomes will be correlated with early molecular response patterns to establish molecular toxicity pathways that can be used to predict apical outcomes in addition to a better understanding of environmental relevance of FLX. This study is part of the EcoToxChip project (@ecotoxchip).

**TP133 Characterizing changes in molecular toxicity pathways to predict adverse outcomes of 17β-trenbolone in adult fathead minnows (*Pimephales promelas*)**

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Metabolites of synthetic steroids that are used in livestock production as growth promoters often are released into surface waters, inadvertently exposing and potentially causing adverse effects to aquatic wildlife. One such steroid is trenbolone acetate, an anabolic steroid used as a growth promoter in beef cattle. Trenbolone acetate is hydrolyzed in the blood into its active form 17β-trenbolone, which acts as an agonist to the androgen receptor. Significant concentrations of 17β-trenbolone are excreted through manure, which can result in exposure of aquatic life such as fish through runoff from feedlots or during field fertilization using manure. Current research suggests 17β-trenbolone impacts reproduction of vertebrates including fish by acting as a potent androgen, thereby decreasing fecundity, altering plasma steroids and changing phenotype. The purpose of this study is to further our knowledge regarding the specific toxicity pathways of trenbolone by identifying key molecular response patterns that may be altered during exposure to 17β-trenbolone in adult fathead minnows (*Pimephales promelas*), and to link these with apical outcomes of regulatory relevance. Specifically, this study will expose male and female adult fathead minnows to 17β-trenbolone for 21d in a flow-through system. Samples will be collected after 4 and 21 days, and will be subjected to 'omics (transcriptomics, metabolomics, proteomics) and apical outcomes (histology, meristic parameters, growth, fecundity, secondary sex characteristics, behaviour) analyses, respectively. We predict this research will identify critical biological endpoints and pathways, which could be utilized to predict apical outcomes across different species and chemicals acting through a similar mechanism of action (i.e. androgens). This study is part of the EcoToxChip project (@ecotoxchip).

**TP134 Time-course *Daphnia magna* metabolomics as a new water quality monitoring tool**

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Environmental monitoring which is essential for environmental decision making has utilized various biological parameters of aquatic species for assessing water quality. The *Daphnia magna* metabolome has been suggested as a potential tool for examining biomarkers of environmental stresses; however, studies for applications in biomonitoring and understanding on dynamics of metabolomic regulation have not been examined in detail. In this study, time-course *D. magna* metabolomics was applied to better understand metabolomic regulation in ambient (non-stressed) conditions. Additionally, to investigate how sensitive and how informative the *D. magna* metabolome in response to aquatic stresses, *D. magna* was exposed to stressful conditions over time. Metabolites reported and annotated by previous studies were selected and the targeted metabolites were quantified using liquid chromatography with tandem mass spectrometry (LC-MS/MS). Under ambient conditions, metabolomic regulation in different development stages, reproduction stages, days and hours were observed. Under stressful conditions, *D. magna* metabolome was monitored in starvation and sub-lethal exposure to a pharmaceutical ingredient and endocrine disruptor. Metabolite dependent periodicity and stability were observed under ambient conditions. Hourly monitoring results indicate the time-dependent metabolomic regulation was tightly organized between individuals. Under stressful conditions, it was revealed that the homeostasis of *D. magna* is highly vulnerable to the exogenous stressors. Additionally, abnormal regulation of *D. magna* metabolome provied mechanistic insights due to changes in water quality. This study demonstrates a strategy to use *D. magna* metabolomics in water quality monitoring and highlights the utility and potential of the time-course metabolomics approach.

## A Celebration of Whole Ecosystem Experiments

**TP135 Accumulation of Silver in Yellow Perch (*Perca flavescens*) and Northern Pike (*Esox lucius*) From a Lake Dosed with Nanosilver**

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A total of 15 kg of silver nanoparticles (AgNPs) was added continuously over two ice-free field seasons in 2014 and 2015 to a boreal lake (i.e. Lake 222) at the IISD-Experimental Lakes Area in Canada. We monitored the accumulation of silver (Ag) in the tissues of yellow perch (*Perca flavescens*) and northern pike (*Esox lucius*) exposed to the AgNPs under environmentally relevant conditions. The greatest accumulation was observed in the liver tissues of pike, and a single pike sampled in the second year of additions had the highest concentration observed in liver of 5.1 µg/g wet weight. However, the Ag concentrations in gill and muscle tissue of both pike and perch did not exceed 350 ng/g. Following additions of AgNP, the Ag residues in fish tissues declined, with a half-life of Ag in pike liver of 119 days. Monitoring using passive sampling devices and single particle ICP-MS during the AgNP addition phase confirmed that Ag nanoparticles were present in the water column and estimated mean concentrations of Ag increased over time to a maximum of 11.5 µg/L. These data indicate that both a forage fish and a piscivorous fish accumulated Ag in a natural lake ecosystem dosed with AgNPs, leading to Ag concentrations in some tissues of the piscivorous species that were 3 orders of magnitude greater than the concentrations in the water. There was evidence of oxidative stress in both perch and pike as a result of exposure to AgNPs.

**TP136 Drone Imagery as a Useful Tool to Survey a Wetland Habitat**

*M.M. Valentine, L.B. Martello, S.J. Luis, Ramboll*

Drone technology was used at a site where investigations from ten years prior identified an endangered species of freshwater shrimp in small, shallow ponds in remote areas across the site. Surveying the site to find these small ponds on foot would be time consuming and difficult given the saturated soil and high, dense vegetation. Also, the likelihood of missing a pond containing shrimp was high given the difficulty of navigating the terrain on foot. Therefore, the use of a drone to identify low lying areas across the site where habitat could support this species of shrimp expedited field efforts. The drone allowed for the identification of vegetation specific to the ponding areas. High-resolution RGB drone imagery was run through supervised and unsupervised image classification with varying numbers of training samples. Image classification results were manually inspected for correctness and compiled to identify the best areas for field biologists to focus their survey. Image classification is effective and relatively quick to process, and provides a means of collecting data efficiently over expansive areas. Drones are also useful when surveying sites where access on foot is problematic such as swamps/marshes, waterways, cliffs, and intertidal habitats that occur intermittently over vast stretches of coastline. Drones are also ideal for identifying habitats with populations of sensitive fauna, such as birds, where disturbance is preferentially minimized. Software is available that allows overlapping aerial photographs to be joined together to create an ortho-rectified photo mosaic. The processing power of some software packages is very sophisticated, allowing three dimensional images to be created using information embedded in aerial images collected using drones. The three-dimensional image can be used to visualize a target whether it is a landform such as a rock outcropping, a water feature, or a nesting area.

**TP137 Ecological Factors Controlling Insect-Mediated Methylmercury Flux from Aquatic to Terrestrial Ecosystems**

*M.M. Chumchal, Texas Christian University; R. Drenner, Texas Christian University / Department of Biology*

The diets of terrestrial consumers can be subsidized by energy and nutrients transported from aquatic ecosystems to terrestrial food webs by emergent aquatic insects. However, the cross-system transport of materials by insects can have a "dark side" because emergent aquatic insects also transport toxic contaminants such as methyl mercury (MeHg) to terrestrial ecosystems (hereafter referred to as insect-mediated MeHg flux). Although ecological factors are known to influence insect emergence and the cross-system transport of energy and nutrients by insects, the ecological factors regulating insect-mediated MeHg flux have been little studied. In this presentation we overview our mesocosm and whole ecosystem experiments examining ecological factors regulating the transport of MeHg out of aquatic ecosystems by emergent insects. We also present a conceptual model integrating the influence of MeHg contamination of ecosystems and ecological factors regulating emerging insect-mediated MeHg flux during seasons when insects are emerging. We propose that the potential for insect-mediated MeHg flux increases with Hg contamination of the ecosystem but that the realized insect-mediated MeHg flux is determined by ecological factors known to regulate aquatic insect community ecology.

**TP138 Plasmid profiling of antibiotic-resistant organisms isolated from hospital effluents discharged into Nworie River, Imo State, Nigeria**

*N. Chiegboka, FUTO / Biology; I.O. Emeka-Nwabunia, FUTO / Biotechnology; M.C. Korie, IMO State Polytechnic Umuagwo, Owerri / Science Laboratory Technology*

The emergence of multiple antibiotic resistance in bacteria and the indiscriminate use of antibiotics contribute to the dissemination of resistant pathogen in the environment. Hospital effluents are potential sources of antibiotic resistant bacteria which if released into the rivers leads to the contamination of the water by the resistant strains which are potential threat to human health as they might have direct access to man or transported from sea animals to man through food.Plasmids are major mechanism for the spread of antibiotic resistant gene in bacteria population. Plasmid profiling is one of the methods used to determine and characterize antibiotic resistance traits in bacteria.In this study, a total of eighteen isolates were screened for antibiotic susceptibility out of which eight (8) isolates were resistant to at least five antibiotics. The isolates were tested against ten (10) different antibiotics using the disc diffusion method while the plasmid DNA were extracted using the TENS extraction method and separated by agarose gel electrophoresis of which four out of the resistant strains had plasmid DNA.

**TP139 The incorporation of elements derived from diluted bitumen into the aquatic food web as reveled by stable isotopic analysis**

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Bitumen mined from the Canadian Oil Sands is highly viscous, so it must be diluted with lighter condensates to facilitate its transport through pipelines, yielding diluted bitumen (commonly referred to as dilbit). Dilbit is transported across Canada in large volumes and puts the sensitive freshwater ecosystems that these pipelines traverse at risk. To better understand the fate and effects of diluted bitumen spills in freshwater, we are conducting a mesocosm experiment at the IISD-Experimental Lakes Area in northwestern Ontario, Canada, called the BOREAL (Boreal Lake Oil Release Experiment by Additions to Limnocorrals) study. This study involves simulating an ecosystem-based pipeline spill in 10-m diameter mesocosms installed in the littoral zone of a boreal lake. Different doses of diluted bitumen are added to the mesocosms, simulating a range of environmentally-realistic spill sizes. Traditionally, stable isotope analyses were used to reconstruct food webs, but this technique may also be a powerful means of monitoring petrogenically sourced elements through a food web following an oil spill. We are evaluating the use of carbon, nitrogen, and sulfur stable isotopes as tools as the isotopic signatures of dilbit differ from that of the aquatic food web. Carbon isotopes reflect diet, nitrogen isotopes indicate trophic level, and sulfur isotopes reflect the relative importance of various diet sources. By combining the findings of these three isotopes, the incorporation of dilbit derived elements may be monitored. We collected samples of microbes, phytoplankton, periphyton, zooplankton, fingernail clams, and fish muscle tissue. Samples will be analyzed by stable isotope ratio-mass spectrometry analysis and compared across dose and time. Stable isotopic analysis of carbon was used to track the Deepwater Horizon oil spill in marine systems and to monitor changes in diet and trophic shifts following the spill, but to date, stable isotopes have not been used as a tool to track oil spills in freshwater ecosystems. The importance of our research stems from its novel application in a freshwater environment, the multi-isotopic approach, and the inclusion of multiple trophic levels within a natural lake food web. Our research will demonstrate the utility and power of applying isotope analysis and the possibility of their application at the site of future oil spills as a tracer of the extent to which organisms can uptake oil derived elements.

**TP140 Understanding the Impact of Vegetation on Water Quality and Biotic Diversity in Agricultural Ditches**

*E. Martin, Arkansas State University / Environmental Sciences; J.L. Bouldin, Arkansas State University / Biological Sciences*

Beginning in the late 1800s, ditch systems in northeast Arkansas were created to drain water from wetlands in the Mississippi River Valley to establish the fertile land for agricultural use. Today, they continue to move water away from fields and discharge into larger streams. This conveyance system is part of the larger Mississippi River drainage basin that feeds into the Gulf of Mexico and contributes sediment and nutrients that exacerbate hypoxic conditions. However, agricultural ditches can be important tools for managing water quality because of their ability to filter sediment and sequester nutrients from field runoff. Aquatic vegetation is an important characteristic of these ditches that influences sediment and nutrient values. Ditches also serve as habitats of ecological importance and act as corridors for aquatic and terrestrial movement between patches within the agricultural landscape. This study investigates the impact of vegetation on water quality and wildlife in agricultural ditches. Ten sites divided between two separate HUC-8 watersheds in northeast Arkansas are measured weekly for three years to monitor water chemistry, turbidity, total suspended solids, total and dissolved nutrients, and chlorophyll *a*. Whole Effluent Toxicity tests are performed quarterly to evaluate the vitality of each site. Bed and bank vegetative coverage is qualitatively characterized at each site and plants are identified to the species level when possible. Spring and summer surveys of macroinvertebrates, amphibians, reptiles, and birds are also conducted. Water quality, sediment, and nutrient variables are compared to vegetation coverage at each site using regression analysis. Sites are then compared between upstream and downstream locations using a Kruskal-Wallis test. Species richness is calculated for each taxa at each site and compared to water quality and vegetative variables. The results of this ongoing study help understand the biological importance of agricultural ditches within the local landscape as well as the in-stream processes that have the potential to improve water quality regionally.

## Pathological Endpoints as Predictive Tools for Relative Ecological Risks of Species

**TP141 Effects of Atrazine on Embryogenesis and Larvae Development of African Catfish, *Clarias gariepinus* (Burchell, 1822)**

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Atrazine is an extensively used herbicide amongst the commonly detected herbicides in freshwater. Early developmental stages of *Clarias gariepinus* are particularly sensitive to water pollution. Freshly fertilized eggs of *C. gariepinus* were exposed to 4µg/L, 8µg/L, 12µg/L, and 16µg/L atrazine through embryogenesis in a static renewal bioassay for 72hours. Seven broad stages of embryogenesis: zygote, cleavage, morulation, blastulation, gastrulation, segmentation, and hatching were observed, photographed and documented live with the aid of a light microscope. The first mitotic cleavage in the control group occurred at 40minutes post-fertilization (pf). However, it occurred at 60minutes pf in the 4µg/L and 8µg/L treatments and 70minutes pf in the 12µg/L and 16µg/L treatments. The first larva emerged at 22 hours at a controlled temperature of 27.0 ± 0.5°C. There was a significant difference ($p < 0.05$) in the hatching rate and percentage deformities between control and treatment groups. Mean total length (TL), mean head length and mean body width of control and exposed larva showed a dose-dependent trend. Larvae deformities such as scoliosis, kyphosis, lordosis, detached yolk, and c-shaped body curvature were observed among treatment groups but were more severe with increasing concentration. These results showed that atrazine delayed embryogenesis and hatching with possible physiological and morphological implications.

**TP142 Oocyte pathology and micro-habitat preference of *T. zilli*, *N. diversus* and *C. gariepinus* within a tropical agrarian catchment**

*A.V. Chukwuka, University of Ibadan / Department of Zoology; O. Ogbeide, University of the Free State / Zoology and Entomology*

Ovarian lesions and defects in three tropical fish species (*Tilapia zilli, Neochanna diversus*, *Clarias gariepinus*) inhabiting a pesticide impacted river was described based on a microscopic analysis. Different pathological alterations were detected in gonads of fishes sampled, including necrotic changes, atretic follicles, intersex, loss of follicular structure, vacuolations. Lesion severity differed across species including intersex and the number of follicles undergoing atresia. Significantly higher number of atretic primary follicles were recorded in benthic species compared to pelagic species. Although different pathological alterations were found in gonads of all species examined, in most cases, it was possible to associate their occurrence of histopathological features with parasitism and ecological behaviour or preferences of the species. Findings suggest that the preference for particular microhabitat may determine ecological risks of a species including, its susceptibility to reproductive toxicity. This study demonstrates the relationship between the uneven distribution of environmental contaminants and the likelihood of relative risks between species occurring within the same habitat.

## Advancing Fate and Effects Modeling and Their Integration to Increase the Relevance and Robustness of Risk Assessments

**TP143 Modeling impacts of climate change: From genomes to phenomes to populations**

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A significant challenge facing biology is understanding how genotypes generate specific phenotypes that influence population-level responses. Addressing this challenge is most urgent today because managing for impacts of climate change requires both understanding and predicting responses to a changing climate within and across levels of biological organization. The processes governing generation of phenotypes are complex and include nonlinear, multivariate and multilevel interactions. Lower level processes can in turn be constrained by population-level properties, which are nonlinear and vary in space and time. Powerful new technologies have impacted virtually all biological disciplines, allowing for unprecedented rates of collection of sophisticated data (e.g., the 'omics revolution). That said, there has been relatively limited progress in our understanding of the mechanistic interrelationships among genes, environment and phenotype and how these interactions emerge at the population level; this limitation constrains the ability to predict responses to a changing climate. To address this challenge, the National Science Foundation has funded a Research Coordination Network (RCN): Predicting vertebrate responses to a changing climate: modeling genomes to phenomes to populations (g2p2pop). The goal of this RCN is to establish a new collaborative network spanning from genomes to populations to facilitate development of novel quantitative approaches to address the urgent challenge of predicting how vertebrate species and populations will respond to climate change-related stressors. It focuses on the interplay between theory and experimentation to develop mechanistic models of acclimation and adaptation to climate change. Collaboration of empirical biologists and mathematical modelers is critical to identifying links and emergent properties across the biological hierarchy, as well as the relative contributions of plasticity and microevolutionary processes to organismal and population persistence in a changing climate. This RCN is populated initially with 28 US and international participants with collective expertise across the spectrum of biological organization and mathematical modeling. All scientists are welcome and actively sought for inclusion in the network.

**TP144 A generalized life-history model for assessing indirect effects of pesticides on fish populations**

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Assessing population-level effects of stressors, such as pesticides, across species is challenging because effects are influenced not only by individual-level toxicity but also by species' life history characteristics, ecology, and the duration, magnitude, and frequency of stressor exposure. Additionally, potential indirect population-level effects of stressor exposure (for example, effects resulting from changes in food availability) add further complexity to the assessment. To address these challenges, we developed a generalized population model for small fishes for assessing potential indirect effects of pesticide exposure on population dynamics. We applied the model to 17 species of darter (Percidae: Etheostomatinae) for which life history and diet data were readily available. The selected darter species (including two species listed under the Endangered Species Act) cover a range of life history strategies and diet compositions within the subfamily. We simulated several scenarios of pesticide exposure to assess how potential impacts of a pesticide on various prey species (invertebrates) included in the diets of selected darter species might affect darter population dynamics over extended time periods. We also investigated correlations between life history characteristics and population responses to the exposure scenarios. This analysis provides a framework for predicting food web mediated effects of pesticides on darter species for which little life history information is available. By combining life history variability in fish with estimates of potential indirect effects of pesticides on their prey, our model can provide a valuable tool for incorporating ecological complexity into the assessment process to quantify population-relevant risks to listed and non-target species of small fish.

**TP146 Spatial and temporal variations of national cropping patterns for higher-tier pesticide exposure assessment**

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Pesticides are used on numerous agricultural crops across the United States to control pests and improve food yield and quality. This presentation focuses on the spatial and temporal aspects of a national scale assessment conducted by the Pyrethroid Working Group (PWG) to characterize the potential for pyrethroids to enter flowing surface waters based on a spatially explicit analysis of crop proximity to surface waters using multi-year data on diverse agricultural production patterns. Standard exposure calculations in the USEPA EFED regulatory risk assessment framework assume that 100% of the area around the water body (the Tier II pond) is cropped and treated, and therefore subject to drift and runoff entry. Over two million catchments within the National Hydrography Dataset (NHDPlus) were characterized using geospatial data to develop national or regional metrics related to potential surface water exposure related to crop proximity for more than 10 crop types. Crop locations were based on five years of data from USDA NASS Cropland Data Layer. Results highlight the variability of cropping density at the catchment scale across different geographies and scales, as well as situations in which density of potentially highly exposing crop (e.g., within 200m of surface water) may not match 'entire catchment' cropping density patterns. Variations in cropping density (as a proxy for potential exposure) across multiple years will be discussed in relation to how this variability may influence exposure estimates. When examining all catchments containing a specific crop, the 90$^{th}$ percentile crop density values (based on the 200m proximity zone) ranged from 1.3% (for vegetables in FL) to 44.4% (for tree nuts in CA). The resulting datasets provide a useful set of metrics across multiple crops which can be applied to pesticide exposure assessments that may need spatially-explicit refinements related to crop-water interactions. Because the crop proximity results are linked to the NHD+ framework, including these specific attributes into other NHD+-based analyses is extremely efficient.

**TP147 A Computational Approach for the Ecological Prioritization of Organic Chemicals in Canada: ERC 2.0**

*M.A. Bonnell, J. Prindiville, M. Jagla, C. Inglis, Environment and Climate Change Canada / Ecological Assessment Division*

In 2016 Environment and Climate Change Canada (ECCC) published the Ecological Risk Classification (ERC), a computational approach used for the re-prioritization of 640 organic chemicals originally prioritized in 2006 as persistent or bioaccumulative and inherently toxic (BT and PT chemicals). The ERC is a multi model risk-based system and was developed to optimize the use of new approach methods (NAMs) in ecotoxicology as well as new developments in fate and exposure science. The ERC is based on the concept of establishing chemical profiles for hazard and exposure. Each profile contains several descriptors that define both the toxicological and exposure spaces used within the profile. The profiles combine data from in chemico, in silico, in vitro and in vivo data to provide several lines of evidence for hazard, exposure and ultimately risk classification for hundreds or thousands of chemicals. The second version of the ERC (ERC2) is under development by ECCC for the prioritization of approximately 12200 organic chemicals on the DSL not prioritized in 2006 as PBT, BT or PT chemicals. New in silico and in vitro tools and data useful for ecological prioritization have become available since 2016 allowing expansion of the hazard rules and descriptors, in particular, from the first version of ERC. Here we present the conceptual model used for ERC2 including the boundaries for data acceptability (e.g., model domain of application, model consensus) and confidence scoring of descriptors used in hazard and exposure classification. ERC2 involves big data and includes information for many hazard and exposure descriptors. It provides ECCC with one approach (substance-by-substance) when considering chemical priorities for post 2020 chemical's management work planning. ERC2 can also be used to aid responses to various regulatory questions or needs outside of substance-by-substance ranking of risk (e.g., identification of potential endocrine active substances, regrettable alternatives, high hazard or exposure concerns, potential research and monitoring priorities).

**TP148 Modelling the fate and distribution of PFASs in the San Francisco Bay**

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Per and polyfluoroalkyl substances (PFASs) are a class of compounds extensively used in food packaging, commercial goods, and aqueous film forming foams used to extinguish fires. Some of these compounds, such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), are highly persistent in the environment and living organisms, and can cause adverse health effects, such as endocrine disruption, immunological suppression, and cancer. Elevated concentrations of PFASs, particularly PFOS, have been observed in San Francisco Bay biota. To properly assess the fate of PFASs in the environment and potential risks to humans and the ecosystem, it is necessary to better understand the long-term trend concentration of PFASs in water and sediments. To address this need, we have developed a long-term mass balance constant rate model for PFASs, using PFOA (whose production was phased-out in the U.S. in 2015) as a model compound. We used sediment and water concentrations reported in 2009 as initial concentration values. We also included as inputs in the model the concentrations measured in wastewater treatment plant effluents and stormwater as external loads, as well as estimated contribution from rivers and atmospheric deposition. Our preliminary results suggest that, if actual external loads remain stable, levels of PFOA would decrease in both water and sediment, reaching values ranging between $10^{-1}$-$10^{-2}$ ng/g in sediment and 4.0 -4.5 ng/L in water by year 2052. The total mass of this pollutant within the estuary would be close to 30 kg, out of which, around 75 % would be present in the water compartment. Validation of these results with actual PFOA sediment concentrations from 2010, 2012, and 2014, showed values within the range of results of the different predictive scenarios.

**TP149 Modelling nitrogen fixation in crude oil polluted oil palm plantation in Owaza, Rivers State, Nigeria; a case study on ecosystem damage**

*A.I. Onwurah, University of Nigeria, Nsukka / Environmental Management; I.N. Onwurah, University of Nigeria / Biochemistry*

Crude oil exploration, mining and urbanization influence the dynamics of the environment and could pose adverse effects on ecosystem integrity. Nitrogen fixation in such environment suggests the presence of diazotrophic bacteria. Hence risk assessments, emergency response decisions, and damage assessments for oil and chemical spills in such ecosystem can rely on or modelled with a relevant biomarker. In situ measurements of chemical concentrations in spill-impacted water or soil may provide insight about chemical transport but may not give the degree of risk or damage to the ecosystem without integration of the biological component. Although a biological receptor component in risk assessment may pose an additional degree of complexity, it is most likely to provide a more realistic assessment in terms of biomarker relevance, time and space. In this work, we propose a nitrogen fixation model for crude oil polluted soil ecosystem based on the case study of the oil spill that took place in Owaza oil palm plantation in Nigeria in 1996. Nitrogen fixing bacterium *Azotobactervinelandii*, a diazotroph is a proven biomarker as it provides an important biological function ($N_2$-fixation) that can be related to an exposure to, or toxic effect of an environmental chemical or chemicals. This biological response can provide qualitative and semi-quantitative information on the quantum of the oil spill, and information on the relationship between $N_2$-fixation and levels of oil pollution in the given oil polluted ecosystem. The biological function F, which corresponds to the entire cellular activity of the diazotroph, is the production of $NH_4^+$ ($N_2$-fixation) by the nitrogenase enzyme complex. The mathematical model governing the entire process ($N_2$-fixation) is based on cybernetics and structured approach to biochemical processes in microbial cells. With this model, an empirical assessment can be made concerning the damage caused to the oil palm plantation ecosystem by the oil spill incident in terms of nitrogen fixation that was compromised, relative to control (site without oil spil incident).

**TP150 Age-dependent Human Elimination Half-lives of DL-PCBs Using the Cross-Sectional Biomonitoring Data in General Population**

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Elimination half-life is crucial in the application of pharmacokinetic model to assess human health risk of PCBs. Previous studies used constant half-lives to estimate PCBs elimination in human body, while mounting studies indicated elimination rate, as well as half-lives, may be affected by age. In this study, 306 general populations were recruited and blood samples were collected in 2012. Meanwhile, dietary structure-based food samples were collected and daily intake of dioxin-like PCB were estimated. The first-order model was employed to simulate the human biomonitoring data, while exponential model, linear model and constant model were used to determine the relationships between elimination rate and age. For people aged 20-50, the average half-life of PCB189 is the shortest (from 0.67 years to 1.23 years), followed by PCB126 (2.98 years to 5.43 years), PCB123 (2.96 years to 5.72 years), PCB157 (4.65 years to 6.66 years), PCB105 (4.87 years to 7.46 years), PCB114 (6.07 years to 9.82 years), PCB169 (6.50 years to 7.87 years), PCB118 (6.90 years to 8.51 years). Half-life values of PCB167 and PCB156 were age-independent, and 8.05 years and10.55 years, respectively. The results show half-lives of high halogenated dioxin-like PCBs are longer than that of low halogenated dioxin-like PCBs except for three congeners of them: PCB189, PCB157 and PCB118. Our results for the first time clarified the effects of age on elimination rate of dioxin-like PCBs. Our research provide an important basis for making accurate health risk assessments for dioxin-like PCBs.

## Human Health and Ecological Risk Assessment of Per- and Polyfluoroalkyl Substances

**TP151 Unique Challenges in Site Specific Risk Assessment at a Receptor-Diverse PFAS Site Investigation Area in Jervis Bay, Australia**

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Environmental risk assessment at PFAS impacted sites continues to evolve and presents unique challenges due to the persistence, ubiquity and uncertain toxic effects associated with this class of compounds. The Australian Department of Defence (DoD) has been at the global forefront of PFAS site investigations and risk assessments, sponsoring robust characterisation at sites around the nation with the aim of informing associated management and remediation options for beneficial project outcomes. Jervis Bay Range Facility (JBRF) and HMAS Creswell (collectively considered within the broader Jervis Bay area) is one of these investigation areas, and is complex in that four habitats (terrestrial, freshwater, estuarine and marine) support both an abundance of ecological diversity in addition to the resident Wreck Bay aboriginal community. This diverse ecology and extensive community use of the land presents a unique challenge from a risk assessment perspective and requires consideration of a myriad of biological trophic levels and exposure pathways beyond what would be required at a less complex location. This project encompassed a comprehensive sampling program for flora and invertebrate and vertebrate fauna from all four biomes to inform both a human health and ecological risk assessment (in development). This case study details some of the unique considerations required for this investigation, including non-standard exposure assumptions and the moving targets inherent in PFAS investigations as a result of evolving knowledge and regulatory guidance. The project will continue to be a valuable addition to the industry's understanding of assessing this difficult class of compounds, as well as providing an extensive characterisation of PFAS in a variety of media to help advance the scientific knowledge base.

**TP152 Comprehensive Analysis of PFAS in Terrestrial, Freshwater, and Marine Food Webs at Jervis Bay, Australia to Inform an Ecological Risk Assessment**

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The Australian Department of Defence (DoD) is in the process of undertaking a national program to investigate PFAS impacted bases around the nation. Jervis Bay Range Facility (JBRF) and HMAS Creswell (collectively considered within the broader Jervis Bay area) is one of these investigation areas, and is complex in that four habitats (terrestrial, freshwater, estuarine and marine) support both an abundance of ecological diversity in addition to the resident Wreck Bay aboriginal community. The Jervis Bay peninsula, upon which JBRF is set, drains into two distinct catchments, encountering the terrestrial and freshwater ecosystem in Booderee National Park before draining into Jervis Bay to the north and Wreck Bay to the south of the peninsula. The diverse and sensitive ecological receptors in the area required a comprehensive sampling program for flora and invertebrate and vertebrate fauna from all four ecosystems to inform a detailed ecological risk assessment (in development). This case study considers the observed accumulation in biota collected from the region in relation to other risk assessments completed to date, and summarises the potential for risk to this diverse ecosystem. Importantly, the current body of evidence does not suggest adverse effects as a result of PFAS exposure in Australian ecosystems. The project will continue to be a valuable addition to the industry's understanding of assessing this difficult class of compounds, as well as providing an extensive characterisation of PFAS in a variety of media to help advance the scientific knowledge base.

**TP153 Occurrence of perfluorooctanoic acid (PFOA) and Perfluorooctane sulfonate (PFOS) in river system and probabilistic hazard risk assessment**

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Levels of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) in surface water and sediment samples from rivers in South Africa were determined using ultra performance liquid chromatography coupled with quadrupole time of flight (UPLC-QTOF-MS). Concentrations of PFOA in surface water ranged from < LOD to 1929.8 ng/l and concentrations of PFOS ranged from < LOD to 1289.00 ng/l. On the other hand, concentrations of PFOA in sediment samples ranged from < LOD to 214.5 ng/g for PFOA and concentrations of PFOS ranged from < LOD to 246.2 ng/g. Level of PFOA and PFOS obtained from this study were subjected to probabilistic hazard risk assessment to predict the possibility of exceeding suggested threshold limits. Results show that PFOA in water as established by United States Environmental Protection Agencies (USEPA) and Department of Water Council (DWC) Germany threshold limits were exceeded, with distributions of 54 and 56 % respectively for Plankenburg River, and distribution of 50 and 52 % in Diep River. While, percentages exceedent for PFOS in Plankenburg River were 56 and 58 % for USEPA and DWC respectively, and percentage exceedence of 50 and 55 % for Diep River. Continuous release of PFOA and PFOS in the river system could leads to elevated levels; hence trigger environmental and hazard risk to the ecosystems.

**TP154 PFAS Elimination in Eggs of Hens Exposed via Drinking Water**

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Background/Objectives Hens that are exposed to per- and poly-fluoroalkyl substances (PFAS) via drinking water, food and soil eliminate/excrete PFAS in eggs. The consumption of eggs from backyard hens has the potential to represent a significant dietary exposure pathway for people living in areas where soil and water have been contaminated by PFAS. However, few studies have been published to quantify the relationship between PFAS concentrations in eggs and environmental media, representing a source of uncertainty for human health risk assessment (HHRA) of PFAS in the environment. The objective of this study was to characterise PFAS concentrations in eggs at a range of PFAS concentrations in the hen's drinking water, and evaluate whether PFAS concentrations in chicken eggs could be estimated based on the hen's PFAS intake from drinking water. Methodology The study involved 119, 30-week-old Hy-Line Brown hens that had started egg-laying prior to introduction to the trial. The hens involved in the study were managed with due regard for their welfare as required under an animal ethics approval. The PFAS examined were perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS), perfluorooctanoic acid (PFOA) and perfluorohexanoic acid (PFHxA). There were four treatment groups for the study, with each group provided drinking water with the same concentration of each of the four PFAS (0.3 mg/L, 3 mg/L, 30 mg/L and 300 mg/L). There was also one control group, where the chickens were provided drinking water with no detectable PFAS. After a 14 day pre-treatment phase to acclimatise birds to the study conditions, the hens were exposed to PFAS in their drinking water over a 61 day treatment phase aiming to evaluate long-term average egg PFAS concentrations likely to be encountered at each treatment concentration. Egg samples and drinking water samples were collected during each phase of the study. Results Key findings were as follows: No changes in health or productivity (e.g. hen's body weight, egg weight, egg laying rate or water consumption rate) were observed in hens from all treatment groups during the study. The amount of PFAS in the edible part of the egg was found to be proportional to the amount of PFAS consumed by hens in their drinking water. PFAS was primarily detected in the yolk of the egg, with negligible amounts in the egg white. PFOS elimination/excretion in eggs was greatest for PFOS, followed by PFHxS, and notably lower for PFOA and PFHxA.

**TP155 Data to support the development of wildlife toxicity reference values for per- and polyfluoroalkyl substances**

*A. Jackovitz, US Army Public Health Center / Toxicology; M. Quinn, Army Public Health Center / Health Effects*

Per- and polyfluoroalkyl substances (PFAS) are compounds manufactured for use in paints, cleaning agents, fire suppressants, non-stick cookware and food containers, and water-impermeable products in general. Concerns about PFAS stem from their ubiquitous presence in the environment, widespread reports of general toxicity, and the resistance of these compounds to degradation. In a range-finding experiment, six PFAS (perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), perfluorohexanesulfonate (PFHxS), perfluorobutanesulfonate (PFBS), 6:2 fluorotelomer sulfonate (6:2 FTS), and perfluorononoate (PFNA) were administered to the white-footed mouse (*Peromyscus leucopus*) for 28-consective days. The goal of the study was to ensure bioaccumulation and sufficient body burden of the chemicals for follow-up reproductive/developmental toxicity studies, which will be used to derive wildlife Toxicity Reference Values (TRVs). In addition to determining steady state dynamics and possible target organs, mortality data were used to develop interim TRVs. Wildlife TRVs and increased understanding of PFAS in general, will assist in the development of appropriate site-specific risk assessment and decisions related to mitigation of exposures and/or future cleanup.

**TP156 Per- and polyfluoroalkyl substance (PFAS) prioritization and risk factors for terrestrial ecological receptors**

*A. East, Towson University / Environmental Sciences; R.H. Anderson, US Air Force / ORD / NCEA; C.J. Salice, Towson University / Environmental Science & Studies Biology*

Per- and polyfluoroalkyl substances (PFASs) remain a complex environmental contamination and management problem. Challenges in evaluating risk of PFASs as both individual compounds and mixtures to ecological receptors lie in the shortage of toxicity data and the need to prioritize relevant PFASs. Environmental sampling data show that most PFASs exist in the environment as complex mixtures. However, these mixtures and, importantly, their parent formulations are not consistent in space nor time so determining the most relevant PFAS(s) for ecotoxicity testing and risk assessment remains critical. We developed a method to help prioritize compounds for testing and risk assessment from environmental sampling datasets. The method explicitly considers environmental concentrations, frequency of detection and sampling, and several metrics related to uncertainty and mixture characteristics. Surface water and soil concentration datasets, respectively, were used to initially develop and then apply the method. We found that PFOS and PFHxS are likely the majority PFASs of concern. Others such as PFOA, PFHxA, and the fluorotelomers (6:2, 8:2 FTS and FOSA) are of concern, but with lower confidence. In order to more explicitly link this exposure profile to an ecologically relevant endpoint, we used PFOS specific toxicity reference values (TRVs) for avian and reptile receptors as threshold effect levels to explore how environmental concentrations may translate to potential risk. We used BSAF values for soil dwelling invertebrates paired with incidental soil ingestion estimates for birds and reptiles to estimate sum PFAS uptake through diet and soil exposure. As the input data for this estimate is a distribution of soil concentrations from PFAS contaminated sites, the resulting distribution of daily intake values can then be compared against TRVs. Results suggest that 8 to 38% of sites would be in exceedance of TRVs, depending on species- and environment-specific factors. Importantly, the likelihood of this intake varies across individual compounds due to varying BSAF values and across locations due to the influence of site-dependent characteristics (e.g. the specific PFAS mixture). In summary, we have demonstrated the application of a method useful in screening a large number of sites and samples to identify priority PFASs, risk drivers, and to help define key uncertainties in risk estimation.

**TP157 PFAAs and EcoRisk: Development of a Hazard Ranking System by Evaluating Functional Groups vs. Chain Lengths as Primary Risk Drivers**

*J.K. Wilhelm, A. Luz, A. Luz, P.E. Goodrum, Integral Consulting Inc.*

Per- and polyfluoroalkyl substances (PFAS) are a vast group of chemicals with varying properties and rapidly developing science. While ecological risk assessment frameworks exist to address emerging contaminants such as PFAS, there are few working examples, and many questions remain regarding appropriate methods to apply when PFAS mixtures are detected at a site. This presentation focuses on perfluoroalkyl acids (PFAAs), which have been detected ubiquitously in the environment, and are expected to persist in biotic and abiotic media and potentially to bioaccumulate. Historically, PFAAs have been classified into groups based on their carbon chain length and associated carboxylate or sulfonate functional group. However, there is growing evidence that variability in relative potencies and bioaccumulation potential warrants a more refined classification system. Toxicological studies useful for ecological risk assessment have focused predominantly on two of the most commonly detected long-chain PFAAs, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). It is now understood that there are important differences in relative potency among PFAAs with the same carbon chain length, in part due to differences in functional groups, but also due to variability in species sensitivities. This presentation will highlight key factors that may explain differences between PFAAs in kinetics and target tissue concentrations. We propose a framework for incorporating such information in a site-specific hazard ranking system for both long- and short-chain PFAAs as they relate to aquatic and mammalian ecological receptors.

**TP158 Applying a "One Health" Approach to the Assessment of PFAS**

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The One Health concept recognizes the interconnected health of the environment, animals and humans and that a transdisciplinary approach can achieve optimal health outcomes by recognizing these interactions. Due to their ubiquitous presence from widespread usage in consumer products, commercial operations, and firefighting, and their environmental persistence, per- and polyfluoroalkylated substances (PFAS) are globally distributed in the environment and detectable levels of PFAS are present in virtually all humans, as well as, terrestrial and aquatic animals. The One Health concept is used to evaluate PFAS in the environment through a review of toxicity information, fate and transport information, sampling data, and regulatory efforts. Based on environmental media and biota concentrations observed in our work and others at several sites around the globe, we illustrate likely exposure scenarios for biota and humans, and highlight some of the current challenges in assessing risks of this unique class of chemicals. An extensive toxicity database has been compiled for PFAS based on over 250 publications, which is being used to help evaluate potential risks to terrestrial and aquatic systems. Site-specific bioaccumulation rates developed using field data demonstrate that for some types of fauna, bioaccumulation of certain PFAS can be significant. Information from our research and that of others indicates that potential exposure pathways of PFAS in soils, sediments, and water to animals and plants are complete, and PFAS is transported through the food chain, presenting an additional exposure pathway to humans as well. Fish tissue PFAS data, for example, indicate potential linkages between ecological and human health suggesting that consumption of fish may be a major source of exposure to these chemicals. When combined with potential intakes through drinking water as well as consumer exposures through household items, the possibility exists for much higher intakes and much longer-term exposure than estimated through environmental media alone. Using a One Health approach will help promote integrated assessments of PFAS as well as promoting discussion of the potential effects at the population level from current and continued exposures to PFAS through environmental media.

**TP159 An Evaluation of Perfluoroalkyl Substances-based Fish Consumption Advisories in North America**

*R.R. Holem, GEI Consultants; J. Newsted, OBG*

Perfluoroalkyl substances (PFAS) are synthetic compounds that are persistent and resistant to degradation in the environment and have received considerable attention with respect to their occurrence and fate in the aquatic environment and biota. Consumption of fish is a known source of human exposure to PFAS, particularly PFOS. As such, PFOS-based fish consumption advisories (FCAs) have been issued in several US states and Canada. Fish tissue PFOS concentrations and the methodology and assumptions (e.g., fish consumption rate) used to develop PFOS-based FCAs will be examined. Case studies of locations with significant PFAS fish tissue data will be presented. Factors such as the water body type (lentic vs. lotic) in which PFAS-based advisories are present, PFAS concentrations across fish species, and species-specific bioaccumulation will be discussed. The extent of PFOS-based FCAs will be compared to that of legacy compounds such as Hg and PCB to assess if PFOS-based FCAs are greater or less restrictive in areas where these compounds have been quantified.

**TP160 ITRC Project Team on Per- and Polyfluoroalkyl Substances (PFAS)**

*Y. Lowney, Alloy, LLC / Health Sciences; L.H. Wilson, Sage Risk Solutions LLC; R. Mueller, New Jersey Department of Environmental Protection / Division of Science, Research, & Environmental Health; V. Yingling, Minnesota Department of Health / Environmental Health; P. Reyes, ITRC*

PFAS are a large and complex class of anthropogenic compounds whose prevalence in the environment have become an emerging, worldwide priority in environmental and human health. Some PFAS are environmentally persistent and bioaccumulative and may pose human health risks. Recent high-profile cases involving human exposure in the United States have further focused both public and regulatory scrutiny on PFAS. The scientific community's understanding of PFAS sources, site characterization, environmental fate and transport, analytical methods, and remediation is growing rapidly. However, there is no central clearinghouse available that presents this information in a manner readily accessible to those other than subject-matter experts. As a result, there is a gap in the broad technical understanding necessary for informed and expedited decisions by regulators and policy makers. In response to this information need, ITRC formed a technical team to produce concise technical resources that will help regulators and other stakeholders improve their understanding of the current science regarding PFAS. This poster will provide an overview of the ITRC Team progress, goals, and specific efforts that have been conducted or are underway under the ITRC PFAS team. These include: Fact Sheets: ITRC has developed a series of fact sheets, each synthesizing key information for one of the following core subjects: Naming Conventions and Physical and Chemical Properties Regulations, Guidance, and Advisories History and Use Environmental Fate and Transport Site Characterization Considerations, Sampling Precautions, and Laboratory Analytical Methods Remediation Technologies and Methods Aqueous Film forming foams (AFFF) to be published in August 2018 Technical and Regulatory Guidance Document: The project team is in the process of developing a technical and regulatory guidance document and an internet-based training course, that address the technical focus areas bulleted above. The guidance document will be a web-based resource for environmental project managers. The document and training will provide links to pertinent scientific literature, stakeholder points of view, technical challenges and uncertainties, and the necessary breadth and depth not given by the fact sheets.

**TP161 Phytoextraction of PFAS: Compounding Exposure Risks?**

*U.K. Vedagiri, AECOM / Design and Consulting; B. Harding, AECOM; H. Loso, AECOM / Remediation*

Phytoextraction of PFAS by way of passive and active plant pumping mechanisms has been documented in both laboratory and field scale studies. This presentation explores plant uptake of PFAS, plausible phytoremediation mechanisms, and considers the ecological merits and liabilities of transferring PFAS into biotic media. Plant uptake of both short-chain and long-chain PFAS compounds is well-documented in both herbaceous and woody vegetation. Phytoextraction is currently under consideration as one element of multi-pronged remediation strategies for PFAS in soil and groundwater where cultivation and harvesting/removal of PFAS-accumulating plant species may be a useful risk management tool. However, the plant uptake behavior may also contribute to ecological risks related to PFAS when herbivorous receptors feed upon vegetation. Field studies of PFAS uptake at contaminated studies reveal differential patterns of uptake and accumulation depending on chemical chain length, functional group, soil and groundwater geochemistry, plant species and plant parts. This study utilizes available project-specific field data and literature to identify the ecological benefits and concerns related to PFAS under phytoremediation and risk management scenarios. Data from soil and groundwater phytoremediation studies using historically-documented plant species and data from various sites are evaluated and presented from human and ecological risk perspectives. These risk-based models can serve as decision trees which support practitioners in evaluating the net benefits of applied phytoextraction at PFAS sites and plant-uptake studies.

**TP162 NMR Metabolomics Study of Arabidopsis Exposure to PFOA and PFOS**

*L. O'Hara, University of Guelph / School of Environmental Sciences; J. Konzuk, Geosyntec Consultants; J. Longstaffe, University of Guelph / School of Environmental Sciences*

Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) are environmental contaminants originating from their use in industrial surfactants, textile finishes and flame retardants. Their widespread use has resulted in these compounds becoming ubiquitous environmental contaminants. The persistence of PFOA and PFOS poses an ecological and health risk due to movement up the food web through bioaccumulation. Non-targeted metabolomics provides an avenue to determine the subtle responses by plants when exposed to these compounds at environmental levels. This poster presents new work exploring the application of non-targeted metabolomics using nuclear magnetic resonance (NMR) as an approach to understanding the affect of PFOA and PFOS contamination on plants. *Arabidopsis thaliana* has been chosen as the model organism, as previous studies have shown signs of oxidative stress at high concentrations of PFOA. Non-targeted metabolomics will provide insight into changes expressed in the metabolism of the plants when exposed to environmentally relevant contamination levels. By employing principal component analysis (PCA), metabolic profiling can be used to determine biomarker metabolites for *A. thaliana* response to PFOA and PFOS contamination. This method could potentially be used on a wide range of species to determine whether they show signs of exposure to PFOA or PFOS contamination at levels that may otherwise be difficult to monitor.

**TP163 Perfluorohexanesulfonate (PFHxS) induced apoptosis of neuronal cells via the altered signaling pathways**

*S. Park, Daegu Catholic University / Pharmacology / Toxicology; J. Yang, Catholic University of Daegu / Pharmacology*

Perfluorohexanesulfonate (PFHxS), one of the major perfluoroalkyl compounds (PFCs), has widely been used in a variety of industrial and consumer applications and detected in humans as well as wildlife. Recently, it is reported that PFHxS levels in the cord blood are associated with birth outcomes in the general population. This raised a great concern over its developmental health effects, including neurotoxic effects. While apoptosis of neuronal cells is a key element in neurotoxicity, its underlying mechanism of PFCs-induced apoptosis remains unclear. It has been reported that AMP-activated protein kinase (AMPK) acts as a key signal molecule in neuronal excitotoxicity as well as providing a neuroprotective function. In the present study, we examined the involvement of AMPK in PFHxS-induced neuronal apoptosis using neuronal differentiated PC12 cells, in an attempt to dissect the signaling mechanism. PFHxS induced significant increases in intracellular [$Ca^{2+}$] via the NMDA receptor and the L-type voltage-gated calcium channel (L-VGCC). The inhibition of $Ca^{2+}$ loading by the NMDA receptor antagonist, MK801 and the L-VGCC blockers, nifedipine and diltiazem significantly reduced PFHxS-induced apoptosis. PFHxS induced sustained activation of AMPK and the inhibition of AMPK activation by compound C and AMPK siRNA significantly reduced PFHxS-induced caspase-3 activity. These results indicate the pro-apoptotic role of AMPK. The activation of AMPK was attenuated by MK801, nifedipine and diltiazem. However, the activation of AMPK was not affected by the ERK inhibitor, PD98059. Likewise, ERK activation was not affected by compound C but was substantially reduced by MK801, nifedipine or diltiazem. This suggests that the activation of AMPK and ERK is regulated by intracellular $Ca^{2+}$ loading in distinct pathways. Taken together, PFHxS-induced neuronal apoptosis is mediated by AMPK and ERK pathways. Our findings provide evidence for the underlying mechanisms responsible for PFHxS-induced neuronal damage, which may contribute to identifying target molecules critical for assessing PFC-related neurotoxicity.

**TP164 Effects of PFAS on Sensitive Life Stages of the Fathead Minnow**

*J.G. Suski, EA Engineering, Science and Technology, Inc., PBC / Water and Natural Resources; C.J. Salice, Towson University / Environmental Science & Studies Biology; M.K. Chanov, EA Engineering, Science, and Technology, Inc., PBC / Ecotoxicology; J. Redifer, M. Jersa, E. Brooks, EA Engineering, Science, Technology, Inc., PBC*

There is widespread and growing concern regarding the environmental effects of Per- and polyfluoroalkyl substances (PFASs). Among the most commonly detected and studied PFAS are perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). Both of these PFASs have been measured in a wide variety of wildlife tissues, drinking water sources and surface and ground waters. To date, the consensus is that PFOS is likely the most toxic PFAS and generally dominates environmental samples. Importantly, these two PFAS infrequently occur by themselves; there are often a multitude of "other" PFASs that co-occur with PFOS and PFOA although for these PFASs, there are considerably less data. Indeed, even for PFOS and PFOA more toxicity data over critical life stages would be useful in fine tuning toxicity and threshold estimates. Hence, an important research objective moving forward in environmental assessment and management of PFASs is to better define the ecotoxicity of relevant PFASs and PFAS mixtures. We have conducted the first of eight toxicity studies that encompass sensitive life stages critical to reproduction and development on a representative freshwater fish, the fathead minnow (*Pimephales promelas*). These are 42-day studies where exposures occur over spawning and then continue for 21-days of the F1 generation. Preliminary results show that PFOS, a predominant PFAS, significantly decreases survival of the spawning adults at 500 ug PFOS/L; moreover, there is a very clear threshold for developmental effects between 250 and 500 ug PFOS/L where survival significantly decreases from 92% to 10%, respectively. These results in combination with peer-reviewed literature show that high but, environmentally relevant concentrations of PFOS can elicit toxic effects over longer-term exposures highlighting the need to evaluate this class of chemicals under chronic exposure durations.

**TP165 The Effects of Dermal Exposure to Poly- and Perfluoroalkyl Substances on Post-Metamorphic Amphibians**

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Poly- and perfluoroalkyl substances (PFASs) are distributed throughout ecosystems globally and have been targeted for regulation due to their persistence in the environment, widespread accumulation in both humans and wildlife, and potential for a variety of adverse effects. While PFAS toxicity has been examined in a variety of taxa (e.g., birds, mammals, fish), research on amphibians is limited. We examined the effects of PFAS exposure via contaminated substrate on the survival and growth of juvenile American Toads (*Anaxyrus americanus*), Eastern Tiger Salamanders (*Ambystoma tigrinum*)and Northern Leopard Frogs (*Lithobates pipiens*). Treatments included perfluorooctanoic acid (PFOA), perfluoroocatane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS), and 6:2 fluorotelomer sulfonate (6:2 FTS) at concentrations of 10, 100, and 1000 parts per billion, and a control treatment. Individuals were uniquely identified to assess percent changes in mass and snout-vent length (SVL) over the 30-d experiment. While there was no influence of PFASs on survival or mass of the species, we found significant effects on SVL. Salamanders exposed to PFASs generally exhibited 5 to 10% greater growth in SVL compared to the control, while frogs and toads displayed the opposite pattern. Effects on SVL growth were strongest with exposure to 6:2 FTS and weakest with PFOA. There was limited evidence for a dose-response relationship for the PFASs. We also applied a residual condition index (RCI) to evaluate relative body condition, with controls as our reference RCI. Salamanders showed decreased RCI for all treatments with the exception of PFOS, frogs decreased regardless of treatment, and toads were highly variable. While additional research is needed to determine the mechanisms underlying the contrasting effects of PFASs on salamanders and anurans, our work demonstrates that PFASs can have sublethal effects on amphibians.

**TP166 Adverse Outcome Pathway-based transcriptional point of departure assessment of the effects of perflorooctanesulfonic acid on zebrafish embryos**

*E.J. Perkins, M.S. Wilbanks, N. Vinas, L. Burgoon, US Army Engineer Research and Development Center / Environmental Laboratory*

Perfluoroalkyl substances (PFAS) such as perflorooctanesulfonic acid (PFOA) have been widely used in industrial and consumer products which has led to wide spread contamination of the environment. Chemical concentration thresholds for adverse effects caused by PFOS are needed for protecting both humans and aquatic species. We examined the effects of PFOA on developing zebrafish embryos to identify potential adverse outcome pathways and chemical thresholds by which PFOA can cause developmental toxicity. We exposed zebrafish embryos from 6 hours post fertilization to 96 hours post fertilization to four different concentrations of PFOS. We then used transcriptional and morphological effects to identify potential adverse outcome pathways (AOP) and effect thresholds for PFOS. Morphological effects observed at high doses included swim bladder malformation, cranial malformations, spinal curvature, and embryo orientation. Functional analysis of gene expression indicated that PFOS caused oxidative stress in embryos with antioxidant genes TXN, TXNRD1 and GSS monotonically up regulated, leading to cell cycle arrest with downregulation of genes involved in DNA synthesis, DNA damage repair, telomere extension and cell cycling. Additionally, there was strong down regulation of genes involved in extracellular matrix production including collagen production and lysyl oxidase function indicating a reduction in extracellular matrix. Over all, at high doses, affected gene functions point to a senescence response. Using genes with monotonic dose-responsive expression along with observed apical effects, we developed an AOP network where PFOS causes oxidative stress, leading to autophagy and degradation of the extracellular matrix, altering embryo notochord development and ultimately leading to malformations and mortality. We then calculated an effect threshold level based on a transcriptional point of departure analysis using gene expression in the AOP network. This approach appears promising in identifying plausible AOPs underlying the effects of PFASs and developing AOP based concentration thresholds below which effects are unlikely to be observed. These thresholds can be useful in risk assessment applications by linking mechanisms of toxicity to adverse effects through AOPs.

## Non-Targeted Analysis: Comparing ENTACT Results and Assessing Informatics Approaches

**TP167 Developing non-targeted analysis techniques for chemical prioritization in environmental water samples**

*A. Batt, USEPA / SED / National Exposure Reseach Laboratory; M.S. Kostich, USEPA / ORD / NERL / EMMD*

The analysis of chemical contaminants and their introduction and transport through the environment has been a major focus of environmental work for years, due to their potential to cause undesirable ecological and human health effects. In order to achieve detection levels needed for environmental protection, analytical methods are usually limited to maximum of around 100 compounds. With many tens of thousands of industrial chemicals in use today, prioritizing which analytes or mixtures of analytes should be targeted for analysis has become increasingly difficult. We are developing non-targeted chemical screening tools to look for a much broader range of possible chemical contaminants present in environmental water samples using liquid chromatography and time of flight mass spectrometry (Waters Xevo LC-QToF). Different instrument conditions are being tested, including a variety of LC columns with different sizes and stationary phases (C18, HILIC), different mobile phases and modifiers. Ideal method conditions and criteria needed for data workflow development, including blank subtraction, retention time monitoring, replicate injections, peak alignment, and sequence set up (with possible sample randomization) are also being investigated. Special attention is also being paid to developing laboratory quality performance measures, as this is currently not a standardized aspect of non-targeted analysis. This includes developing protocols for using extraction performance standards, daily instrument performance standards to monitor retention times, sensitivity, and mass calibrations, and optimizing the number of replicate sample injections for different applications. Future work will apply these methods to prioritize which chemicals are responsible for biological activity in complex environmental mixtures, such as municipal wastewater and surface water.

**TP168 Improving non-target analysis by HPLC-ESI/HRMS in the context of the EPA collaborative trial project (ENTACT)**

*B. Ng, Florida International University / Chemistry and Biochemistry; N. Quinete, Florida International University / Analytical Chemistry; P.R. Gardinali, Florida International University / Chemistry & Biochemistry and SERC*

Advancements in analytical chemistry, especially mass spectrometry, have redefined the field of contaminant detection. Majority of approaches for screening of environmental contaminants target individual chemical compounds or classes of chemical compounds using highly specific analytical methods. Despite their ability for low level detection and quantification, novel contaminants or transformation products which may still pose a risk to humans and wildlife are often overlooked by these methods. Non-targeted analysis requires no prior knowledge of compounds and has become very popular in the last couple years for the determination of new and emerging contaminants, or transformation products. The ENTACT sample set was created using about 1200 standard chemical substances from the EPA's ToxCast library to produce 10 liquid mixtures containing 100-400 compounds each. In addition, three types of samples – house dust, human serum and silicon bands – were each spiked with "unknown" standard mixtures to assess different matrix effects. The detection of "unknown" chemicals from the ENTACT samples was carried out by acquiring spectral information using a Q-Exactive Orbitrap mass spectrometer operated at 140,000 resolution power, two ionization modes, and two polarities both under MS and $MS^2$. Subsequent data processing and identification of unknown compounds was performed using the Compound Discover 2.1 software. The blinded results of this study showed that our method identified 2310 features in the standard mixture samples. In the different matrix tested, the highest number of compounds were identified in the silicon band samples, which showed a total of 314 features in the samples (n=2). The human serum samples were found to contain the least amount of compounds; total of 58 features. The performance of our non-target method will be assessed by comparing the results after unblinding.

**TP169 Application of on-line SPE coupled with LC/QToF mass spectrometry to analysis of target and non-target contaminants in water**

*L.K. Kanagy, E.T. Furlong, W.T. Foreman, USGS / National Water Quality Laboratory; D.W. Kolpin, USGS / Central Midwest Water Science Center; P.M. Bradley, USGS / South Atlantic Water Science Center*

High-resolution mass spectrometry (HRMS) increasingly has been used to explore in environmental matrices the presence of previously unidentified compounds beyond those contaminants that are routinely monitored using targeted chemical analytical methods. Identified non-target chemicals might constitute exposure to under-investigated "emerging contaminants" and warrant their inclusion as target analytes in future monitoring programs and effects studies. The USGS National Water Quality Laboratory is using liquid chromatography with quadrupole time-of-flight mass spectrometry (LC/QToF) both for non-target analysis and to confirm the presence of target compounds determined by target-analyte methods (e.g., by LC/tandem mass spectrometry, LC/MSMS). One potential limitation of most LC/QToF systems is that detection limits (DLs) typically are one order of magnitude or more higher than those obtained by targeted LC/MSMS methods (DL ranges of 1-100 ng/L are common for analytes such as pesticides and pharmaceuticals by MSMS). This occurs in part because mass spectrometer dwell times in targeted LC/MSMS methods are sequentially focused on the compounds of interest, while QToF analyzers collect all ion signals concurrently, limiting sensitivity and dynamic range, especially in complex sample matrices. This limits the applicability of the LC/QToF for both targeted and non-target analyses, especially for direct injection analysis of matrices like groundwater and drinking and surface waters with low or sub ng/L contaminant concentrations. To lower DLs, we have applied on-line solid-phase extraction (OLSPE) to pre-concentrate analytes from the sample matrix or sample extract prior to back elution and direct introduction onto the analytical column of the LC/QToF operated in positive or negative electrospray ionization modes. Analyte load and elution conditions were tested using C8 or Oasis® HLB SPE columns (Waters Corp.) and variable pH (3, 7, 10) to identify several optimum OLSPE conditions using a subset of target analytes (pesticides, pharmaceuticals, industrial chemicals) that might be representative of the chemical functionalities of many possible non-target contaminants. The OLSPE LC/QToF procedures were applied to drinking and surface water samples collected for studies conducted by the USGS Toxic Substances Hydrology Program and to test samples from EPA's Non-Targeted Analysis Collaborative Trial (ENTACT). Advantages and limitations of OLSPE are presented.

**TP170 Comparison of workflow techniques for high-resolution mass spectrometry data reduction and prioritization of known, suspected, and unknown contaminants**

*E.T. Furlong, L.K. Kanagy, C.J. Kanagy, W.T. Foreman, USGS / National Water Quality Laboratory*

The rapid expansion and application of high resolution-mass spectrometry (HRMS) analysis techniques to environmental samples has resulted in many different approaches to better characterize complex mixtures of organic contaminants present in environmental samples. Typically, analysis of a single sample can result in thousands of potential contaminant candidates having unique mass spectral and retention time characteristics. Critical components for any HRMS work flow include reducing the inherent complexity of the acquired data by rapidly screening and marking contaminants previously known or suspected to occur (from targeted analyses of the same samples or from similar studies) so that greater effort can focus upon the more complex task of unambiguously identifying unknown contaminants for further assessment and quantitation. There are multiple iterative approaches available to complete these components, but comparison of performance between different approaches has been less frequently evaluated and assessed. We present results from the application of several different approaches to identify known and expected organic contaminants from a common set of environmental and quality control samples. Environmental results for stream samples were obtained from polar organic compound integrating sampler (POCIS) samples, which provide a concentrated extract of great chemical complexity. All POCIS were deployed concurrently in a surface stream reach in the Northeast United States, and, after retrieval, processed identically. POCIS extracts also were analyzed using a targeted HPLC/MS/MS method that determines 109 pharmaceuticals at low ng/L concentrations, providing data for a specific set of compounds found in these same samples. We compared a standard metabolomics software suite (MarkerLynx from Waters Corporation), an open-source data reduction tool (EnviMass, supported by Loos Computing), and the multivariate analysis capabilities of the UNIFI software data processing suite (Waters Corporation) combined with marker screening tools developed in-house using Excel (Microsoft). Preliminary results indicate considerable overlap between candidate marker sets produced by the different software tools, with multiple approaches reinforcing identification of markers associated with specific sample types, and that use of multiple tools provides complementary information for identifying and confirming known, suspected. and unknown contaminants.

## Environmental Risk Assessment

### TP171 A Proposed Method of A Priori Selection of Brood Area to be Monitored in a Brood Termination Study

*A. Warmkessel, S. Ma, G. Kunkel, Smithers Viscient / Carolina Research Center; M. Tomka, Smithers Viscient*

*Apis mellifera* has been identified by the EPA as "an appropriate surrogate for evaluating pesticide risks to bees and insect pollinators in general." As per EPA and OECD guidelines, brood termination is a measure of colony health that is implemented particularly when observing the effects of an insect growth regulator. The guidelines are vague on how brood area that will be monitored throughout the study should be selected. In addition, ways of following brood area over time have grown from using physical markers on the frames to using digital photography of entire frames. The lack of input from the guidelines as well as the change in how data is collected should prompt us to consider "rules" in how brood is selected prior to data collection. These rules take into account the inconsistency between colonies as well as theoretical location of the brood nest within the colony. Putting these selection rules in place would eliminate the need to select brood at the time of, or after, data collection in a subjective manner. This method will be evaluated in the summer of 2018.

### TP172 Acute chronic ratios in algal toxicity

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Algal toxicity studies are required by regulatory agencies for a variety of purposes including classification and labeling and for chemical risk assessment. As algae are often the most sensitive taxonomic group (approximately forty percent of the time) and often drive risk assessments. Understanding acute:chronic ratios for algae can be informative for some assessments. Applying acute:chronic ratios is an approach commonly used when one or the other statistical endpoint is unavailable. Complexities to develop acute:chronic ratios for algae exist including a lack of universally agreed upon algal inhibition endpoints, the evolution of experimental designs over time and by different standardization authorities, and changes in statistical emphases (use of regression-based versus hypothesis test-based effect conclusions) which means there is considerable variance among current ACRs. In most regulatory frameworks, the accepted acute:chronic ratio for algae, fish and invertebrates remains 10 regardless of species, chemical type or mode of action. However, information indicates that the acute to chronic ratio for algae is lower than that for fish and invertebrates thus making algae chronic endpoint extrapolation significantly more conservative than necessary. To date, experimental data for developing a globally accepted algal acute:chronic ratio has been limited largely due to data availability. Using the newly developed ecological Thresholds of Toxicological Concern database and a well-structured internal algal database in our possession, we compiled acute and chronic toxicity data on X algae species and Y chemicals. Information was probed for growth rate, yield, and final cell density endpoints focusing primarily on 72 and 96 h duration studies. Comparisons of comparable acute and chronic data (e.g., growth rate) as well as mixed comparisons (e.g., growth rate and final cell density) were used to judge available acute and chronic relationships. Linear regressions of various model permutations were used to establish proposed acute:chronic ratios for various data combinations. Acute:chronic ratios were consistently around 4 regardless of dataset used or combination of endpoints. We propose an acute chronic ratio of 4 for algal toxicity and outline the conditions under which the ACR can be applied as well as indicating areas for further research and improvement.

### TP173 Addressing Issues in Assessing Environmental Fate & Transport for Challenging Chemical Classes

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Determining the fate and transport of chemicals in the environment is central to evaluations of ecological and human health risk under the Toxic Substances Control Act and other EPA authorities. USEPA, Office of Pollution Prevention and Toxics (OPPT)'s fate assessors in the Risk Assessment Division (RAD) have developed approaches for assessing the fate and transport of new chemicals to reflect the best-available science. Several categories of new chemicals such as per/polyfluoros compounds, nanomaterials, and metal oxide complexes have posed challenges in environmental fate assessment due to their poorly understood environmental behavior, and physical-chemical properties. In many cases, interpretation of chemical fate and transport of these new chemicals is hindered by a lack of data on the parent compound and degradants in environmental media. Additionally, the level of uncertainty in fate characterization can be attributed to the difficulty to conduct laboratory testing on the parent materials, as factors such as low to no solubility, slow or sometimes decades-long degradation reactions, and various analytical challenges complicates their environmental fate evaluation. The presentation will describe the needs, as well as the methods of RAD to assess environmental fate pathways of the above mentioned chemicals. Specifically, it will identify and address the issues associated with: 1) the behavior of these chemicals, 2) identification of degradation rates, degradation intermediates, and final products, 3) the limitations of using predictive models to assess these types of chemicals, and 4) the deficiencies in the current fate test guidelines and modifications developed in conjunction with the stakeholders. This presentation will also show various methods and continuing efforts undertaken by OPPT/RAD to resolve these issues, for instance: 1) development of in-house chemical class-specific databases for identification of known-fragments of intermediates, by-products, and end-products from the degradation of the parent compounds, 2) development of knowledge banks based on information gathering from well-established research and data of previously submitted new chemical cases to support assessments of chemicals that are outside the scope of predictive models, 3) continuous refinement of fate assessment methodologies, and 4) working collaboratively with new chemical submitters to improve testing methods, protocols, and guideline modifications.

### TP174 An approach to estimate hexabromocyclododecane (HBCD) trophic transfer in terrestrial food chains

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Hexabromocyclododecane (HBCD) is a brominated flame retardant largely used in the construction industry in expanded polystyrene foam (EPS) and extruded polystyrene foam (XPS). HBCD is classified as a persistent, bioaccumulative, and toxic (PBT) chemical with the potential to undergo biomagnification in aquatic ecosysstems. Specifically, HBCD has been demonstrated to be toxic to both aquatic and terrestrial organisms in both lower and higher trophic levels, however most of the available data is for aquatic food webs; there is a knowledge gap regarding the potential for HBCD to biomagnify in birds of prey that do not consume fish. Further, biomagnification factors derived from mammal-to-bird food chains have been found to differ from those derived from bird-to-bird food chains, suggesting accessibility to different types of prey may affect HBCD trophic transfer to birds. An approach for estimating the potential of HBCD trophic transfer between terrestrial mammals and predatory birds will be presented. Specifically, data from laboratory feeding studies with rats will be used as a surrogate to estimate HBCD body burdens in small terrestrial mammals (e.g., mink and rodents). Birds of prey, such as American kestrel, typically consume small terrestrial mammals. Data from kestrel ecological toxicity studies will be used to evaluate whether the consumption of small terrestrial mammals will exceed exposure concentrations (e.g., biota monitoring data) that have been shown to result in reproductive toxicological effects in American kestrel. *Disclaimer:*

*The views expressed in this abstract are those of the authors and do not necessarily represent the views or the policies of the U.S. Environmental Protection Agency.*

### TP175 Arsenic exposure, profiles of urinary arsenic species, and polymorphism effects of glutathione-s-transferase and metallothioneins

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This study assessed the effects of polymorphic variants of gutathione-S-transferase and metallothioneins on profiles of urinary arsenic species. Nineteen wells of groundwater for human consumption from the municipalities of Margarita and San Fernando, Colombia were analyzed for concentration and the lifetime average daily dose (LADD) of arsenic. Specific surveys on 101 individuals aged between 18 and 75 years old were applied to collect demographic information and other exposure factors. In addition, GSTT1-*null*, GSTM1-*null*, GSTP1-rs1695 and MT-2A-rs28366003, genetic polymorphisms were evaluated either by direct PCR or PCR-RFLP. We examined arsenic speciation through concentrations of urinary metabolites related with exposure using Atomic Fluorescence Spectrometry: inorganic arsenic (InAs) ($As^V$ and $As^{III}$), monomethylarsonic acid ($MMA^V$) and dimethylarsinic acid ($DMA^V$) and Total urinary As (TuAs). Also were calculated the ratios of arsenic metabolites as indicators of the metabolic capacity; primary methylation index (PMI) and secondary methylation index (SMI). The effects of polymorphisms were tested using a multivariate analysis, adjusted by potential confounders. The arsenic concentrations in groundwater were on average 34.6±24.7 µg/L greater than US Environmental protection Agency guideline for arsenic (10 µg/L). Was observed a significant correlation among LADD of arsenic and TuAs ($r=0.69$; $p=0.000$). %InAs was associated with GSTP1, age, BMI, alcohol consumption, LADD and the interactions GSTP1*Age, GSTP1*BMI, GSTP1*alcohol consumption ($R^2=0.43$; likelihood-ratio test, $p=0.000$). PMI was associated with GSTP1, GSTM1, sex, BMI, alcohol consumption and the interactions GSTP1*GSTM1, GSTP1*Sex, GSTP1*BMI and GSTP1*alcohol consumption ($R^2=0.20$; likelihood-ratio test, $p=0.007$). GSTP1 (AG+GG) homozygotes/heterozygotes and LADD of arsenic could increase %InAs and decrease the calculation of PMI ratio in people exposed to low and moderate levels of arsenic from drinking groundwater. However the explanatory models stronger showed the participation of some covariates that could to influence the effects of the polymorphisms on these exposure biomarkers to arsenic

### TP176 Assessing of dietary exposure risk to methylmercury in seafood in Taiwan

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Being surrounded by the ocean, Taiwan enjoys rich and abundant aquatic resources. Seafood is the main source of dietary exposure to methylmercury (MeHg). Samples of fish, shellfish, cephalopods, crustaceans and algae were purchased from the major fish port, traditional wet markets and supermarket across Taiwan. Some of the raw sampled products were cooked. We analyzed the total mercury concentration of 151 raw samples and 141 cooked samples, and convert it to methylmercury concentration. Pelagic fish, inshore fish, other fish, shellfish, cephalopods, crustaceans and algae MeHg average concentration in raw samples was 0.61 mg/kg, 0.11 mg/kg, 0.05 mg/kg, 0.02 mg/kg, 0.02 mg/kg, 0.03 mg/kg, 0.01 mg/kg, respectively. Furthermore, MeHg average concentration in cooked samples was slightly high than the raw samples. Methylmercury concentrations are high in shark, swordfish, and tuna, but the average concentrations did not exceed the regulatory standards of Taiwan. The ratio of aquatic products that exceeded the limits for methylmercury was not high.

### TP177 Atmospheric Deposition and Toxicity Assessment of Polycyclic Aromatic Hydrocarbons (PAHs) in Ambient Air of Yenagoa and its Environs

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Atmospheric levels, distribution and toxicity of sixteen (16) USEPA priority PAHs in ambient air of Yenagoa communities were evaluated using USEPA method 8015B. Among the PAHS determined, benzo(b) fluoranthene was found to be the most abundant compound at all sites totally 116 16.06 $gKg^{-1}$ or 29.09%, followed by benzo(a) anthralene (85.0 14.02 $gKg^{-1}$; 21%), Chrysene (51.0 7.50 g /Kg; 12.88%), Fluoranthene (49.0 4.7 $gKg^{-1}$; 12.37%, phenanthrene (21.0 7.5, $gKg^{-1}$; 5.3%) anthialene (9 2.81; 2.3%)etc. Some of the pollutants like benzo(a)pyrene, dibenzo (a.h) anthracene and benzo (g,h,l) perylene are found to be low or negligible in most of the locations. Generally, the PAHs concentrations of PAHs determined at different sites in increased markedly in this order: AQBJ>AQOG>AQTR> AQIM> AQOT>AQGU. Multivariate analysis including principal component analysis (PCA) and Correlation Matrix (CR) showed that three major factors (industrial activities including gas flaring, traffic/commercial activities and biomass combuastion) were responsible for the concentrations measured and sites with similar activities correlated well. Factor 1 with Eigen value of 3.7268 accounted for 62.1% variance, factor 2, 17.7% and factor 3, 17.7%. Hierarchical cluster analysis identified two main clusters: AQOT location used as the control and others. Similarly, source diagnostic ratios revealed that PAHs load in AQOT is of pyrogenic origin and others mainly petrogenic origin. Finally, risks posed by these pollutants to the inhabitants of these communities were calculated and the results expressed in terms of hazard quotient (HQ) (Pyrene 1.0 anthracene 0.87; fluoranthrene O.35; napthanlene 0.1) and 'excess life cancer risk' (ELCR) (dibenzo (a,h) anthracene $1.27x10^2$; benzo(a)anthracene $14.6x10^{-1}$; indeno (1,2,3-cd) pyrene $4.2x10^{-1}$; benzo(k)fluoranthene $1.7x10^{-1}$ and chrysene $2.1x10^{-3}$) for non-cancer PAHs and carcinogenic PAHs respectively.

### TP178 Changes of exoskeleton surface roughness and chitinase gene expression in the mud crab following the antifouling biocide irgarol

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Irgarol is a common antifoulant present in coastal sediment. The mud crab *Macrophthalmus japonicus* is one of the most abundant of the macrobenthos in the costal environment, and its exoskeleton has a protective function against various environmental threats. We evaluated the effects of irgarol toxicity on the exoskeleton of *M. japonicus*, which is the outer layer facing the environment. We analyzed transcriptional expression of exoskeleton, molting, and proteolysis-related genes in the gill and hepatopancreas of these exposed *M. japonicus*. In addition, changes in survival and exoskeleton surface characteristics were investigated. In the hepatopancreas, mRNA expression of chitinase 1 (*Mj-chi1*), chitinase 4 (*Mj-chi4*), and chitinase 5 (*Mj-chi5*)increased in *M. japonicus* exposed to all concentrations of irgarol. *Mj-chi1* and *Mj-chi4* expressions from 1 to 10 µg $L^{-1}$ were dose- and time-dependent. Ecdysteroid receptor (*Mj-EcR*), trypsin (*Mj-Tryp*), and serine proteinase (*Mj-SP*) in the hepatopancreas were upregulated in response to different exposure levels of irgarol at day 1, 4, or 7. In contrast, gill *Mj-chi5*, *Mj-Tryp*, and *Mj-SP* exhibited late upregulated responses to 10 μg $L^{-1}$ irgarol compared to the control at day 7. *Mj-chi1* showed early upregulation upon exposure to 10 μg $L^{-1}$ irgarol and *Mj-chi4* showed no changes in transcription in the gill. Gill *Mj-EcR* presented generally downregulated expression patterns. In addition, decreased survival and change of exoskeleton surface roughness were observed in *M. japonicus* exposed to the three concentrations of irgarol. These results suggest that exposure to irgarol induces changes in the exoskeleton, molting, and proteolysis metabolism of *M. japonicus*.

**TP179 Comparative Evaluation of Agricultural Pesticide Usage in Knights Landing and Capay Valley, California**

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Knights Landing (KL) and Capay Valley (CV) are both communities in Yolo County surrounded by agricultural fields, but CV specializes in organic crops. KL residents expressed concerns about elevated exposure to potential carcinogens due to proximity of agricultural pesticide applications and they invited UC Davis researchers to further investigate. This study quantifies the amount of applied pesticides, carcinogenicity of pesticide active ingredients, crop-specific pesticide application, and method of application longitudinally from 2011-2015 in KL and CV. Pesticide application reports were retrieved from the Pesticide Information Portal (PIP) from the California Department of Pesticide Regulation. The weight of pesticide applied was normalized to total acreage in the 2 KL and 3 CV zip codes included in the PIP data. Carcinogenic chemicals were identified using the USEPA Standard Evaluation of Pesticides for Carcinogenic Potential and the IARC Monographs. We found that four times more total pesticides by weight and eight times more carcinogenic pesticides by weight were applied in KL compared to CV. Therefore, KL community exposures to agricultural pesticides are potentially higher than CV, supporting the concerns raised by the KL community members.

**TP180 Consideration of Skin Sensitization in Identifying Preservatives for the USEPA's Safer Chemical Ingredients List**

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The USEPA Safer Chemical Ingredients List (SCIL) identifies safer ingredients using robust, transparent and publicly available criteria for several functional use classes including preservatives. Chemicals meeting these criteria are listed on the SCIL and can be used in Safer Choice-certified products. Preservatives play a critical role in consumer products by guarding against microbial contamination and degradation. They pose a challenge for formulating with safer chemistries due to their inherent bioactivity, which may produce unintended toxicity. Recent news reports concerning preservatives in consumer products has focused on cases of dermal allergic reactions. Currently, there are 31 preservative chemicals listed on the SCIL, which are allowed in Safer Choice-certified products. They are marked based on overall confidence in the hazard profile, with either a green circle (N = 22, verified low concern), green half-circle (N = 2, low concern but needs additional data), and yellow triangle (N = 7, some hazard concerns but represents best in class for the function). This marking reflects an overall finding based on 10 hazard endpoints, including but not limited to skin sensitization. A curated database of experimental data for preservatives was created using publicly-available secondary sources describing clinical studies in humans and experimental animal model of skin sensitization (e.g., local lymph node assay [LLNA], guinea pig maximization test). Primary sources were consulted when discrepancies necessitated an in-depth review. Analog data were used to evaluate endpoints with experimental data gaps. In some cases, a weight of evidence evaluation was used to address data sets with mixed results (e.g., positive clinical data, negative animal data). The majority of green circle preservatives are of low concern for skin sensitization. The half-green preservatives currently lack a reliable dataset to evaluate skin sensitization. The yellow triangle preservatives generally have some positive skin sensitization data, but represent best in class when compared to other preservatives with known sensitization issues. As per Safer Choice criteria, preservatives in Safer Choice-certified products are allowed at the lowest possible effective level. The results of this evaluation demonstrate how SCIL status can be used to select safer preservatives for consumer products.

**TP181 Current State of Cleanup Levels and Approaches for Petroleum-Contaminated Sites**

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Risk evaluations for petroleum release sites present complex and unique challenges to site managers, risk assessors, regulators and other stakeholders. Risk characterization based on total petroleum hydrocarbon (TPH) concentration measurements in different environmental media may be used for risk-based corrective actions at petroleum release sites. However, chemical compositions of petroleum hydrocarbon mixtures are complex and subject to change over time due to fate and transport processes. While methods to characterize risk for individual constituents are well accepted, methods to assess cumulative risk posed by the multitude of petroleum-related compounds typically included under the term "TPH" have yet to be widely accepted and employed. This is compounded by inconsistencies between published guidance for the risk-based assessment of TPH-related compounds and requirements for expensive laboratory tests that might not be available in many areas of the country. Better guidance is needed to help states develop consistent methodology for establishing risk-based cleanup levels and for establishing and approving methods for risk-based corrective action. A state survey was conducted by the Interstate Technology and Regulatory Council (ITRC) TPH Risk Evaluation at Petroleum-Contaminated Sites Team. A total of 53 complete responses were recorded from 44 states (a few with multiple programs), Washington D.C., and Puerto Rico. The goal of the survey was to collect information on state agencies' use of TPH data within their regulatory programs and to help develop the ITRC technical and regulatory (Tech Reg) guidance that describes best practices for evaluating TPH risk. This presentation will highlight the various approaches used by different states and programs, analysis of the survey results, and its implementation in the ITRC Teg Reg guidance.

**TP182 Data Gap Filling with ECOSAR and Application to K-REACH Compliance for Environmental Risk Assessment**

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USEPA/OPPT developed ECOSAR model and still updated for hazard and risk assessment to chemicals under the TSCA. It is a QSAR model and used for data gap filling of lack or no experimental measured data chemicals in TSCA. This model consider chemical class based on structure as well as toxicity mechanisms. As a screening level tool, it is also used to other chemical regulations like EU REACH, South Korea K-REACH. We applied ECOSAR (v2.0) to estimate aqatic toxicity to fill data gaps to establish SSDs of 20 organic chemicals. However, it is an old tool firstly developed in 1990s. Therefore, it is need to check whether ECOSAR still shows estimation capacity considering new toxicity data. We investigated ECOSAR estimation capacity before applying to the K-REACH compliance. We targeted 35 organic chemicals as the lack of toxicity data of 20 chemicals and compared the each chemical geometric value to ECOSAR predictions. We collected toxicity data at ECOTOX database and calculated species mean toxicity value with SD(standard deviation). We applied ECOSAR model if we found data gap in K-REACH compliance like risk assessment. Model applicability was evaluated by comparing model output and experimental data, where we focused on the SD of experimental data. For regulatory purpose, we consider lower toxicity range (high toxicity than experimental) like -1SD, -2SD, and -3SD, which are conservative range. However, upper and lower toxicity range are considered for toxicity estimation. As a result, ECOSAR fish toxic estimation of 18 chemicals show appropriate for regulatory purpose. Among 18 chemicals, 4 chemicals show highly conservative (between -2SD and -3SD or -3SD outline), but these are not suite for toxic estimation (between +2SD and +3SD or +3SD outline). 5 chemicals are moderately conservative (between -1SD and -2SD) and these are moderately toxic prediction (between +1SD and +2SD). Then 9 chemicals are in -1SD, which are not suite for regulatory purpose (slightly conservative), but fish toxic estimation is highly predictive (in +1SD). Organic chemical class of that 9 chemicals are Neutral organic(3), Phenol(2), Halide(3), and Aniline(1). As a conclusion, ECOSAR may not appropriate to some chemical class for regulatory purpose. However, we investigated limited number of groups and chemicals. Therefore, in further study, we are going to expand the number of chemical groups and associated chemicals to clarify model applicability to K-REACH in risk assessment

**TP183 Defining Substances of Concern for Environmental Risk Assessment based on European Guidance**

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Consumer product manufacturers evaluate the hazard profile of ingredients used in their products to ensure safety and meet regulatory requirements. The Guidance on the Biocidal Products Regulation (BPR) released by the European Chemical Agency (ECHA) in October 2017, defines parameters for identification of Substances of Concern (SOC) in biocidal products. In general, these parameters include hazard and concentration criteria. Among other purposes, SOCs are selected for environmental risk assessments of mixtures. An overview of how this guidance impacts environmental risk assessment of a biocidal product will be provided. A process will be proposed to screen chemicals based on their toxicological properties to determine the likelihood of being flagged as an SOC. Additionally, applicability of the guidance for chemical selection when assessing mixture toxicity in non-biocidal products will be analyzed.

**TP184 Development and application of a multidimensional density dependent matrix population model for Atlantic killifish (*Fundulus heteroclitus*)**

*D.H. Miller, USEPA / Mid-Continent Ecology Division; B.W. Clark, D.E. Nacci, USEPA / Atlantic Ecology Division*

Modeling exposure and recovery of fish and wildlife populations after stressor mitigation serves as a basis for evaluating population status and remediation success. The Atlantic killifish (*Fundulus heteroclitus*) is an important and well-studied model organism for understanding the effects of pollutants and other stressors in estuarine and marine ecosystems. Herein, we develop a multidimensional density dependent matrix population model for Atlantic killifish that analyzes both size class structure and age class structure of the population simultaneously over time. This population modeling approach emphasizes application in conjunction with field monitoring efforts (e.g., through effects-based monitoring programs) and/or laboratory analysis to link effects due to chemical and/or nonchemical stressors to adverse outcomes in whole organisms and populations. We applied the model to investigate population trajectories for Atlantic killifish exposed to 112, 296, and 875 pg/g of 2,3,7,8-tetrachlorodibenzop-dioxin with effects on fertility and survival rates. For each exposure concentration of 2,3,7,8-tetrachlorodibenzop-dioxin, the corresponding plots of total population size, population size class structure, and population age class structure over time were generated. For example, exposure to 875 pg/g of 2,3,7,8-tetrachlorodibenzop-dioxin resulted in a 13.1% decline in population size after 2 years, a 19.9% decline in population size after 5 years, and a 27.9% decline in population size over 10 years with plots of all size classes and age classes exhibiting declines. The present study serves as an example of how multidimensional density dependent matrix population models are useful tools for ecological risk assessment because they integrate effects across the life cycle, provide a linkage between endpoints observed in the individual and ecological risk to the population as a whole, and project outcomes for future generations.

**TP185 Development of an EPA GIS Mapping Tool for Accessing Estimated Water Chemistry Parameters to Support Criteria Implementation**

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EPA has developed a mapping tool for states, tribes, and other users to locate estimated water chemistry parameters for their input into a biotic ligand model, multiple-linear regression, or hardness based metals-criteria model when there is insufficient water chemistry data available at a given site. These estimated parameters are based on data contained in draft "Biotic Ligand Model Missing Parameters Technical Support Document" (EPA 820-R-15-106), now updated with additional information. This product is intended to support the development of criteria that rely on the use of water chemistry parameters (temperature, pH, dissolved organic carbon, alkalinity, calcium, magnesium, sodium, potassium, sulfate, and chloride) by providing a user-focused and visual representation of the information found in the draft Biotic Ligand Model Missing Parameters Technical Support Document. Users will be able to locate a site's estimated parameters at the Level 3 ecoregion and can search for a location via NWIS station number, NPDES permit number, location name or coordinates, and ecoregion. Users can click on an area of the map to access a site's estimated water chemistry data. Measured water quality data, such as geochemical ions (Cl, $SO_4^{-2}$, Na, Mg, Ca, K, carbonate/alkalinity), DOC, conductivity and stream order were retrieved from the United States Geological Survey National Waters Information System (USGS-NWIS) stations for rivers and streams between 1984 and 2018. GIS data was used to locate each NWIS sampling location at the Level 3 Ecoregion, based upon the latitude-longitude spatial coordinates. GIS was also used to assign the Strahler stream order to each NWIS sampling location. Measured values for the water chemistry parameters are used as attributes of each NWIS station and the station is plotted on US map. DOC concentration are from the *National Bioaccumulation Factors Technical Support Document* (EPA-822-R-03-030). EPA prefers the use of current, site-specific, water chemistry data when available but has provided this tool for instances when sufficient data to run a model is lacking.

**TP186 Disrupting effects of antibiotic sulfathiazole on developmental process during sensitive life-cycle stage of *Chironomus riparius***

*K. Park, Chonnam National University / Department of Fisheries and Ocean Science; I. Kwak, Chonnam National University / Faculty of Marine Technology*

Antibiotics in the environment are a concern due to their potential to harm humans and interrupt ecosystems. Sulfathiazole (STZ), a sulfonamide antibiotic, is commonly used in aquaculture and is typically found inaquatic ecosystems. We evaluated the ecological risk of STZ by examining biological, molecular and biochemical response in *Chironomus riparius.* Samples were exposed to STZ for 12, 24 and 96 h, and effects of STZ were evaluated at the molecular level by analyzing changes in gene expression related to the endocrine system, cellular stress response and enzyme activity of genes on antioxidant and detoxification pathways. STZ exposure induced significant effects on survival, growth and sex ratio of emergent adults and mouthpart deformity in *C. riparius*. STZ caused concentration and time-dependent toxicity in most of the selected biomarkers. STZ exposure leads to significant heat-shock response of protein genes (HSP70, HSP40, HSP90 and HSP27) and to disruption by up-regulating selected genes, including the ecdysone receptor gene, estrogen-related receptors, ultraspiracle and E74 early ecdysone-responsive gene. Furthermore, STZ induced alteration of enzyme activities on antioxidant and detoxification responses (catalase, superoxide dismutase, glutathione peroxidase and peroxidase) in *C.riparius*. By inducing oxidative stress, antibiotic STZ disturbs the endocrine system and produces adverse effects in growth processes of invertebrates.

### TP187 Evaluating *E.coli* exposure to anglers of the Pine River (Michigan)

*H. Wilson, Alma College / Environmental Studies; A.D. Harwood, Alma College / Environmental Studies / Biology*

Former research conducted by the Alma College Department of Environmental Science and the Michigan Department of Environmental Quality has confirmed the presence of thermo-tolerant fecal coliform bacteria including *E. coli* in the Pine River, a tributary of the Chippewa River in Gratiot County, MI. Although signage recommends limited contact with the water, fishing from the Pine River is common practice. Since the presence of *E. coli* and other fecal coliform bacteria has the potential to cause human illness, it is important to determine if contact with water via fishing activities can expose anglers to *E. coli*. A study conducted in 2016 found that these bacteria accumulated on caged hatchery fish in the river, particularly after rain events. The presence of bacteria on native fish, however, was unknown. The current study addresses the presence of thermo-tolerant *E. coli* on resident Pine River fish, as well as evaluates the potential for these bacteria to occur on the hands of recreational anglers. Multiple locations along the river north of the Alma dam were sampled for the presence of *E. coli*. Angled fish and hands of volunteer anglers were swabbed at various time intervals to confirm the presence of these bacteria on the fish and on the hands of anglers. In the laboratory, the bacteria were expelled from the sample swabs and incubated prior to quantification. Fecal coliform bacteria and *E. coli* were present on both the resident fish and the hands of anglers. Fecal coliform bacteria occurred on 100% of the participating angler's hands, and nearly 80% had *E. coli* present on their hands regardless of their interaction with a fish. Therefore, anglers fishing in the Pine River may be exposed to potentially harmful bacteria.

### TP188 Evaluating Environmental Circumstances Representing Critical Conditions for Metals Criteria Applying the BLM – Copper as a Case Study

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The USEPA Health and Ecological Criteria Division evaluated how water physiochemical conditions that affect metal bioavailability relate to stream flow by pairing corresponding measured 2007 copper Biotic Ligand Model (EPA-822-R-07-001) input parameters and stream flow. Relationships between streamflow and parameters that influence metal bioavailability may identify potential high-risk scenarios (i.e., "critical conditions") where metals are relatively bioavailable and toxicity reduction capacity is limited. Paired measurements of stream flow and BLM input parameters (i.e., calcium, magnesium, sodium, potassium, chloride, sulfate, sulfide and carbonate/alkalinity, pH, DOC, temperature, conductivity) were collected from the US Geological Survey National Water Information System (NWIS) for dates between 1984 and 2018. Data for hardness, stream order, presence/absence of dischargers, level III ecoregion, season, weather conditions, and the spatial coordinates of each sampled station were also collected. BLM input parameters were used to predict instantaneous water quality criteria (IWQC), as a surrogate measure of copper bioavailability, that could be paired to stream flow measurements. Paired IWQCs and stream flow measurements for each sampling location were analyzed to explore site-specific relationships between stream flow rate and copper bioavailability (measured as IWQC) using least squares regression. The relationship between stream flow rate and IWQC was negative for 68% of locations, indicating metal bioavailability was greater at higher stream flow rates. However, copper was also highly bioavailable (as indicated by relatively low IWQC values) across a range of stream flow rates, suggesting additional factors besides stream flow may influence the presence of ligands that affect copper bioavailability. Further analysis is underway to assess how season, stream order, ecoregion, land use, and presence/absence of point sources influence the binding ligands which, in turn, affect copper bioavailability.

### TP189 Evaluating Environmental Risk on an Inundated Dam Construction Staging Area

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A baseline human health/ecological risk assessment is being conducted as part of the remedial investigation (RI) for a former construction staging area in the Big Cliff Reservoir on the North Santiam River, Oregon, USA. The Site is a former work area used to support the construction of Big Cliff and Detroit Dams through 1953. The area is now submerged within the Big Cliff Reservoir following the commissioning of the Big Cliff Dam. Legacy wastes associated with historic construction debris were evaluated as potential sources of sediment contamination. Surface and subsurface samples were collected from 31 randomly selected stations and 53 locations targeting areas with visible signs of contamination (e.g. debris, staining). Based on land use history and preliminary data, samples were analyzed for a metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and other SVOCs. RI results indicated that while there was not widespread contamination across the site, there were locations with concentrations of selected metals, PAHs and PCBs above ecological screening levels; human health COPCs were limited to antimony, arsenic, and lead. Contaminant distribution was highly localized, with different chemical suites elevated above screening levels at different locations throughout the site. These localized areas of contamination are likely the result of specific industrial releases that occurred prior to inundation while the site was terrestrial. This limits the likelihood for diffuse lateral contamination typical of more traditional sediment contamination. Additionally, the substrate is highly compacted sand and gravel limiting sediment redistribution across the site. Due to the unusual nature of the site and the localized contaminant distribution, a traditional risk assessment approach using sitewide 95UCL values overestimated risk. Rather the risk assessment was conducted using a step-wise approach, selectively removing stations with the highest concentrations of COPEC/COPCs from consideration based on the assumption that sediment represented by these samples would be remediated or removed. This site-specific approach allowed for the identification and removal of localized and bounded areas of increased contamination that were driving risk, informing and limiting the scope of the feasibility study. This is an approach that may have application for other similar former dam construction staging areas that have been subsequently inundated.

### TP190 Evaluation of the occurrence of fluoroquinolones drugs in river water by liquid chromatography

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Antibiotics are an important class of pharmaceuticals that have been found in water samples and fluoroquinolones (FQs) are an example of this group. Even in low concentrations, they are able to impact the aquatic system because may further increase resistance of pathogenic bacteria. In view of this problem, this study aims to investigate the presence of FQs drugs such as levofloxacin (LEV), ciprofloxacin (CIP), enrofloxacin (ENR), sarafloxacin (SAR) and norfloxacin (NOR) in water samples from Anil River, located in Sao Luis, Maranhao, Brazil. The research was developed at Federal Institute of Maranhao, using samples collected on May and December 2017 from river in different days and in two places (A and B), according to National Water Agency (ANA) recommendations, stored in amber glass bottles and refrigerated at 2 °C for 24h. Before being analyzed, the pH were adjusted to 3 with $H_3PO_4$, afterward the samples were filtered and prepared by solid-phase extraction process with HLB® Oasis cartridges. The obtained sample extract was dried using nitrogen and then reconstituted in mobile phase used on chromatographic process. The analyses were performed on a Shimadzu LC20-AT high performance liquid chromatograph equipped with a Shimadzu RF-20A fluorescence detector, using a Luna C18 column (250mm x 4.6 mm;

5 μm), flow rate 1.2mL/min, temperature column at 35 °C, excitation wavelength of 280nm and emission wavelength of 450nm, mobile phase was methanol and buffer (0.04M $NaHPO_4$, pH 3) in gradient mode. Two FQs (ENR and SAR) were not found on May and December samples but all revealed contamination by at least one of others in a range of concentrations 15.8-100.6 μg.L-1 (LEV), 11.4-112.7 μg.L-1 (CIP) and 10.3-67.7 μg.L-1 (NOR). LEV was not found on place A, NOR was detected in all samples and CIP was present in May on both places and only on place A in December. The presence of LEV, CIP and NOR antibiotics confirms the contamination on Anil River by domestic sewage, because these drugs are widely used in several types of human infections. The absence of ENR and SAR probably is due to they are exclusively used in veterinary medicine and activities involving animal husbandry are not be so prominent in the collection region. The results demonstrates the importance of effective actions against contamination on rivers by domestic sewage, since they could have compounds such as fluoroquinolones, that may cause a strong negative impact to aquatic organisms.

**TP191 Exposure assessment of environmental phenols by cosmetic products to Korean adolescent girls**

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Cosmetics usage of the Korea adolescent girls is increasing, and the average age of use is decreasing. Environmental phenols are suspected endocrine disrupting chemicals (EDCs) and widely used as a preservative in cosmetics and personal products. This study was conducted to analyze exposure levels of environmental phenols such as 6 parabens (MeP, EtP, PrP, BzP, BuP, and HeP), triclosan, and 4 benzophenones (BP-1, BP-2, BP-3, and, BP-8) in urine to adolescent girls (aged between 13-17 years old, and n=123). We recruited from middle and high schools in Korea. In addition, the participants in the intervention study (n=84) were instructed not to use all cosmetics for 48 hours, except cleansing products to identify the factors that contributed to the exposure source or exposure more precisely. The detection rates and mean concentrations of urine samples were MeP (100%, 75.8 ng/mL), EtP (100,58.4), PrP (90, 11.2), BuP (26, 0.2), BzP (29, 0.01), HeP (4, 0.2), triclosan (71, 1.0), BP-1 (99, 0.6), BP-2 (6, 0.1), BP-3 (55, 1.1), and BP-8 (5, 0.02). The EtP showed higher concentrations, whereas benzophenones and triclosan had lower levels than other countries. In the intervention study, the mean concentrations of the 3 parabens (MeP, PrP, and, BuP) in the urine decreased by 17, 41, and 13%, respectively, whereas the mean concentration of EtP, BzP, and triclosan increased after the intervention. Among participants whose exposure levels decreased after the intervention, the concentration of BP-3 in urine decreased by 26%, and the exposure level of EtP was reduced by up to 72%. As a result, we found that cosmetics contributed to exposure to environmental phenols.

**TP192 Exposure to heavy metals in informal workers in Cartagena, Colombia**

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Metals such as lead and cadmium some elements that are used as part of the great forces that move the world in applications including batteries, electronic components, and other important industrial and technological uses. However, exposure to heavy metals is a public health problem, the concentration in blood and other biological fluids are currently the main bioindicators. In Colombia, there is an important informal or artisanal worker population which is vulnerable to poisoning with heavy metals because it does not use the appropriate protection elements and these workers did not have environmental education. The main routes of entry to the body are the respiratory, digestive and percutaneous. High exposure to lead and cadmium can cause hematological disorders, toxic effects on the central nervous system, toxic changes and the function of various organs. The objective of this study is to determine the lead and cadmium levels in blood (PbLB) and (CdLB) in informal worker including fishermen, welders, painters, battery recycler, and automobile mechanics, and electronic technicians. To achieve this purpose, 277 blood samples were collected from informal worker over 18 years of age with their respective informed consent in Cartagena, Colombia. PbLB and CdLB were quantified by graphite-furnace atomic absorption spectrophotometry (GF-AAS) with Zeeman background correction. Hematological parameters, lipid profile, and glycaemia were also determinate in blood samples.PbLB and CdLB correlates with the year in experience and weight, height, body mass index, as well as evidence in the basophilia peripheral blood smear. These results shown that most of the informal workers have considerable lead concentration above 5 μg /dL. The average concentrations in μg/dL ±standard deviation (range) were of PbLB in battery repairers: 7.1±16.8(0.1-72.8), fishermen: 11.3±13(0.4–53.7), electricians technicians: 9.1±16.1(0.4-53.3), mechanics: 9.2 ± 16.3(0.4-51.3), painter 4.3 ± 4.1 (0.9 -11.7), and welders : 3.8 ± 1.2(1.9-8.0), however the average concentration in μg/L of CdLB were in battery repairers: 1±0.8(0.1-3), fishermen: 1.5±0.9(0.14–5.7), electricians technician: 2.2±1,7(0.1-5.0), mechanics: 2.3 ± 16.3(0.3-7), painters 1,3 ±1,4 (0,2-5), and welders : 1.5 ±1.6(0.4-12.8). Therefore, it is concluded that, e-waste recycling activities release heavy metals into the atmosphere including air, soil, dust etc., which causes adverse health effects on the workers.

**TP193 Historical Trends of Polychlorinated Biphenyls in Chesapeake Bay Fsh and Implications for Human Exposure**

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The Chesapeake Bay watershed represents an important national resource for industry, commercial fishing, and recreation spanning six states and the District of Columbia. Polychlorinated biphenyl (PCB) exposure through fish consumption from the Bay continues to pose a risk to human health despite the ban on commercial PCB use in 1979 and remediation efforts for historically contaminated sites throughout the watershed. The objectives of this study were to: 1) provide historical perspective on PCB body burden in fish caught in the Chesapeake Bay watershed; 2) determine whether PCB body burdens in fish decreased over the 16-year sampling period; 3) evaluate the possible impacts to human health through fish consumption from the Chesapeake Bay. Fish tissue concentrations were measured from 1999 to 2016 as part of the Maryland Department of the Environment's fish monitoring program. White perch, channel catfish, and striped bass were selected for comparison based on the availability of data, frequency of sampling, and importance as both a commercial and recreational fish species. Data were compiled and sorted by drainage basin, species, and sampling year, resulting in the selection of seven drainage basins of interest to assess historical trends. Total PCB body burdens for individual fish composites ranged between 2.48 and 2,936 μg/kg with annual average values ranging between 8.52 and 1,522 μg/kg. Based on the results of our study, less than half of the assessed drainage basins showed statistically significant decreases in fish body burdens, with significant numbers indicating body burdens above the 39 μg/kg threshold for increased risk to human health via subsistence fishing.

**TP194 Honey bee risk assessment: Are two larval studies better than one?**

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After the publication of the Guidance for Assessing Pesticide Risk to Bees (USEPA, PMRA, CDPR 2014), USEPA began requesting a suite of honey bee Tier 1 laboratory toxicity studies to support registration and the registration review process of new and currently registered crop protection chemicals, respectively, including the 8-day acute, single exposure and the 22-day chronic, repeat exposure larval toxicity studies. For the acute larval study design (OECD Test Guideline 237), the primary goal is to determine the $LD_{50}/LC_{50}$ following a single exposure to a test chemical (on Day 4) and mortalities are evaluated on days 5-8 of the larval developmental phase. In contrast, in the 22-day chronic larval study design (OECD Guidance No. 239), honey bee larvae are exposed to the test chemical on Days 3-6 with mortality recorded on Days 4-8 during the larval phase, on Day 15 during the pupae phase, and on Day 22 during adult emergence. Given the 22-day larval study design covers all phases of honey bee brood development up to adult emergence and the larval $LD_{50}/LC_{50}$ can be calculated, the European Food Safety Authority (EFSA) prefers this study design over the acute single exposure study design. However, USEPA requires the acute, single exposure study to calculate a dose-based endpoint (i.e., $LD_{50}$) that can be incorporated into the current screening-level risk assessment model (i.e., BeeREX). There are several complications in using the acute larval study design in a risk assessment including: 1) a true daily dose cannot be determined given that provisioned diet is not entirely consumed within the 24-hour period, 2) food consumption is not consistent over the larval development period and 3) the single dose exposure does not represent any realistic exposure scenario that might occur to larvae in a honey bee colony. A preliminary review of $LD_{50}/LC_{50}$ endpoints from both study designs indicate that similar endpoints are derived based on dose (i.e., $LD_{50}$s) for both study types but lower endpoints (greater sensitivity) are typically derived in the repeat exposure design when based on concentration ($LC_{50}$s). We propose that the acute, single exposure study design should not be required for the Tier 1 risk assessment and that the risk to bee larvae should be determined from the test concentration rather than dose using the 22-d larval study.

**TP195 Household Pesticide and Heavy Metal Measurement in Knights Landing and Capay Valley, California**

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Background: Environmental exposures in small farming communities of Yolo County are not well quantified. Monitoring data, epidemiologic studies, and qualitative studies suggest potentially high residential exposures to carcinogens in Northern Yolo County. Our community-based participatory research project was developed with steady community oversight to address gaps in exposure data. We quantified levels of pesticides and metals in household dust and tap water supplied by private wells in both Knights Landing and Capay Valley. Knights Landing is a conventional farming area and the comparison community, Capay Valley, is a predominantly organic growing region. We expect to detect both pesticides and heavy metals in the tap water and dust from these communities with higher concentrations in samples from Knights Landing than Capay Valley. Methods: Pesticide application data obtained from CA Pesticide Information Portal for 2011 to 2015 was used to identify peak application periods of possibly carcinogenic pesticides applied in these communities. Household dust and tap water samples from 5 homes with private wells in each community were collected during the peak Spring pesticide application period. In dust and water, pesticide concentrations were measured using both targeted and non-targeted GC-qTOF-MS and LC-qTOF-MS analysis and heavy metal concentrations were measured using ICP-MS. Consumer water quality reports for public water systems were obtained as a comparison for private wells in the area. Results: Residents in 90% of homes sampled participated in agricultural work, including one pesticide applicator. All homes were adjacent to agricultural fields. Our analysis of pesticide application data indicates ~10 fold more pesticide application in the area near Knights Landing area relative to Capay Valley with peaks in Spring planting season and Fall harvest season. Arsenic, chromium VI, and mercury contamination are of concern in the public water systems of both communities. Conclusions: Drift of pesticides applied to adjacent fields, occupational exposures, and the movement of pets may contribute to the household dust levels of pesticides. Water quality concerns are universal in both communities based on heavy metals from aquifers and pesticide runoff into surface aquifers. Water quality is especially concerning for the abundant unmonitored, unregulated private wells. Funded by UC Davis Environmental Health Sciences Center P30 ES023513 and T32 HL007013.

**TP196 Immunotoxicity of oil sands process-affected water (OSPW) and its fractions in mammalian immune cells**

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Mining of unconventional crude oil sources such as the Northern Alberta oil sands produces large volumes of OSPW. Characterization of OSPW and its associated toxicity is a formidable challenge due to its complex and variable nature. Broadly, OSPW can be physically separated into organic (OSPW-OF) and inorganic (OSPW-IF) fractions. Naphthenic acids (NAs), phenols, and polycyclic aromatic hydrocarbons are major components of OSPW-OF, while heavy metals including mercury, lead, and arsenic, as well as salts make up OSPW-IF. Most research examining OSPW toxicity has focused on the organic fraction and NAs indicating them as the major toxic component. Previously our lab has developed and used a series of cell-based bioassays to compare whole (unfractionated) OSPW and OSPW-OF, finding that whole OSPW was significantly more toxic at NA concentrations from 10-18 mg/L, when compared to equivalent NA doses of OSPW-OF. Here, we extended the use of our in vitro bioassays to examine immunotoxic properties of OSPW-OF and OSPW-IF alone or in combination. Specifically, a murine macrophage cell-line was used to determine cell viability as well as antimicrobial responses following exposures to whole OSPW or its fractions. Viability trends were similar between OSPW-IF and whole OSPW, with both causing ~90% reductions at 17 mg/L (or volume equivalents for OSPW-IF) and producing LC50 values of ~13 mg/L. Only comparatively high NA concentrations (>100 mg/L) significantly affected cellular viability following OSPW-OF exposure. Production of antimicrobial products (e.g. reactive nitrogen intermediates) was significantly impaired in macrophages exposed to 10 mg/L whole OSPW or OSPW-IF (VE), there was no effect in cells exposed to the same doses of OSPW-OF. Preliminary assessments of the relative contributions of OSPW-OF and OSPW-IF to whole OSPW toxicity has implicated OSPW-IF as a major contributor to trends observed in altered viability and depressed antimicrobial product release by macrophages. Together, these results suggest that at NA concentrations < 20 mg/L, inorganic components play a significant role in the observed immunotoxicity of OSPW.

**TP197 Incorporation of Ecosystem Goods and Services into Ecological Risk Assessment**

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Linking the benefits of the natural environment to the health and wellbeing of people has become increasingly important. Identifying, evaluating, and characterizing ecosystem goods and services (EGS) is one way to better understand this human/environment linkage. Within the USEPA Superfund and RCRA hazardous waste site cleanup processes, ecological risk assessment (ERA) is the mechanism by which threats to non-human ecological receptors are evaluated. Sometimes it is difficult to determine or communicate the benefits provided to people by the identified ecological receptors. We believe for some cases, incorporating EGS methodologies and tools into ERAs can be a useful approach to link the benefits to people provided by the environment. Depending on the circumstances, EGS can be incorporated throughout the ERA or during selected steps.

**TP198 Influence of physical parameters in human health risk assessment in areas contaminated by PAHs**

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In Brazil, health risks resulting from exposure to chemical compounds have been addressed in 2009, when federal Resolution CONAMA 420 was published. Despite this, in 2015 only three out of 26 Brazilian states followed the Resolution. The reasons for this are varied, but the one that stands out is the cost of environmental studies for site investigation. Understanding which parameters for the transport of contaminants and human health risk assessment (HHRA) are the most relevant and require more studies is essential for a broad comprehension of the case with optimization of invested resources. The objective of this study was to identify the physical parameters that exert greater influence on the results of human health risk in a scenario of contamination by polycyclic aromatic hydrocarbons (PAHs). From a case study of an area contaminated with PAHs a HHRA was performed to characterize the health risk to future workers who will occupy the study area. In view of the results obtained, a sensitivity analysis of the physical parameters of the studied area hydraulic conductivity, porosity, hydraulic gradient, water depth and fraction of organic carbon (FOC) was carried out. Risk calculations were performed using the Risk Based Corrective Action methodology and sensitivity analysis was performed using the one-at-a-time method. Parameters were varied positively and negatively, at intervals of 25%. Simulations were performed for four different exposure pathways (direct contact with soil, direct contact with groundwater, indoor and outdoor inhalation of vapors) and three distances from the source of contamination. Results showed that the physical parameter that exerted greatest influence on the results of human health risk was FOC. The variation of this parameter impacted the results for all exposure pathways, except for direct contact with the soil. It was possible to observe that the sensitivity to the variation of FOC values varies according to the distance of the source of contamination. The farther the source, the lower the sensitivity of FOC variation; and the higher the FOC the lower the risk by indoor and outdoor vapor inhalation and direct contact with groundwater. The results also pointed out the importance of soil porosity in the results of human health risk. The higher the porosity, the greater is the risk of indoor and outdoor inhalation of vapors. On the other hand, the higher the porosity, the lower the risk resulting from direct contact with water.

**TP199 Is there Value in Revising the Avian TRV for Vanadium Using Dose-Response Modeling with Existing Data?**

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Risk to avian species from exposure to vanadium (V) is often identified during screening ecological risk assessments, particularly for special-status species which are evaluated at the no-effect level. This is primarily a factor of the low avian ecological soil screening level (Eco-SSL) derived by the USEPA of 7.8 mg/kg dw. USEPA notes this value is well below typical background concentrations in the U.S. (median of 55 mg/kg eastern U.S. and 75 mg/kg western U.S.). The low no-observed-adverse-effects-level (NOAEL) toxicity reference value (TRV) used to calculate the avian Eco-SSL was 0.344 mg V/kg-bw/day, based on juvenile chicken growth. Approximately 95 percent of the avian survival, growth, and reproduction studies used to develop the Eco-SSLs were based on chickens. A push away from NOAEL and low adverse effects levels (LOAELS) towards the use of effects thresholds calculated from dose-response models is well documented. We explore whether using effects thresholds calculated from the same datasets used for NOAELs and LOAELs (where possible) would change the outcome of the Eco-SSLs. Data were compiled from the Eco-SSL dataset and additional studies conducted since the development of the Eco-SSL. Dose-response models from these published studies were used to estimate 10 percent effect dose ($ED_{10}$) and 20 percent effect dose ($ED_{20}$) thresholds. TRVs from modeled $ED_{10}$s and $ED_{20}$s were consistently higher than the TRV for the Eco-SSL, and were in the range of the geometric mean of the NOAEL values for growth and reproduction (reported in the avian Eco-SSL), which was calculated at 1.19 mg V/kg bw/day. For example, a log-logistic dose response model developed by combining V dose and egg production data from 15 chick studies resulted in $ED_{10}$ and $ED_{20}$ (and 95 percent confidence limits) of 2.0 (1.6-2.3) and 3.1 (2.7-3.5) mg V/kg bw/day. If these effects levels are used to recalculate the avian Eco-SSL, the resulting values would be 44 (36-53) and 72 (62-79) mg/kg, respectively, which is still in the range of background. We concluded that using the modeled $ED_{10}$ or $ED_{20}$ results would yield a scientifically defensible, protective, and more realistic Eco-SSL, but in the absence of data for more representative avian receptors (i.e., non-chicken species), the revised values would have limited effect in resolving the largest source of uncertainty associated with the Eco-SSL for V.

**TP200 Monitoring and Risk Assessment of Etofenprox Residue in Oriental Melon**

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This study was conducted to monitor the residue of etofenprox in oriental melon and to assess its risk for human health. This study was carried out on three different regions and the pesticide was uniformly sprayed onto the crop at ratio of about 2.1 L/10 $m^2$ (a.i 8%) with three times at intervals of 7 days. The crops were harvested at 0, 1, 3, 5, 7 and 14 days after spraying. The samples collected were divided into two specimens (peelings and flesh) and then each sample was analyzed using LC-MS/MS after pretreatment. Analytical method was validated prior to analysis of samples, showing that method limit of quantitation (MLOQ) was 0.01 mg/kg and the results of recovery with three levels (MLOQ, MLOQ×10, MLOQ×50) ranged from 87.9% to 97.5% and relative standard deviation (RSD) was low enough to confirm the precision of analytical procedure at three different concentrations. Biological half-life was 4.8 days and most of the pesticide remained in peelings part. Based on these results, a risk assessment was calculated using a percentage of acceptable daily intake (%ADI) based on both values of EDI (Estimated Daily Intake) and ADI. %ADI was less than about 3.5%, indicating that residue level of etofenprox at harvest was considered to be safe for human health.

**TP201 Multiple lines of evidence applied for a realistic Toxic Substances Control Act ecological risk evaluation for D4 using environmental monitoring data**

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D4 (octamethylcyclotetrasiloxane) is a high-production-volume cyclic volatile methyl siloxane with a wide range of industrial and consumer applications. A robust aquatic risk evaluation for D4 was conducted using environmental data (D4 concentrations in water, sediment, and tissues) collected under a nation-wide monitoring program facilitated under a consent agreement with the U.S. Environmental Protection Agency (EPA). The ecological risk evaluation conducted was consistent with the principles outlined in the EPA's *Guidance to Assist Interested Persons in Developing and Submitting Draft Risk Evaluations under TSCA*. The evaluation examined multiple lines of evidence (LoEs) to determine the risks from D4 to aquatic receptors in rivers and streams in the United States in the mixing zones downstream from municipal wastewater treatment plant (WWTP) discharges and discharges from manufacturing, processing, and/or formulating (MPF) facilities after onsite wastewater treatment. The risk assessment LoEs consisted of 1) comparing D4 concentrations measured in water and sediment to toxicity thresholds derived from laboratory studies; 2) comparing D4 concentrations measured in fish and benthic invertebrate tissue to a critical target lipid body burden threshold; 3) comparing fugacity-based chemical activities between laboratory-derived toxicity thresholds and measured environmental concentrations; and 4) assessing benthic macroinvertebrate community structure and habitat suitability. The approach taken moves beyond a standard deterministic hazard quotient risk approach to incorporate more advanced methods for risk prediction, including use of distributions rather than conservative point estimates of exposure to obtain a realistic view of the probability of harm. This risk evaluation concluded that there is negligible risk to water column and sediment receptors from current levels of D4 discharged from MPF facilities after onsite wastewater treatment or from municipal WWTPs that may treat a mix of industrial and consumer wastewater.

**TP202 Murine lung epithelial cell cytochrome P450 monooxygenase dependence of naphthalene toxicity in an in vitro model**

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Background: Naphthalene (NA) is a ubiquitous compound found in the environment. NA is a known mouse lung carcinogen, but the carcinogenicity may not be relevant to humans and other species with lower NA metabolic potential in the airways. We developed an in vitro model of lung-liver crosstalk to test the influence of local airway epithelial cell and liver hepatocyte metabolism on the generation of toxic NA metabolites. Hepatic contribution to NA toxicity in the airway cells is expected to be significant when the local airway metabolism is impaired. Methods: Airway cells from the tracheas of wild type (WT) or *cyp2abfgs-null* female mice were cultured in Transwell inserts at the air-liquid interface (ALI). Primary mouse trachea cells were allowed to differentiate for 4 weeks. Cells were exposed to NA in media with liver microsomes from WT or *cyp2abfgs-null* female mice. Liver microsomes were boiled to inactivate P450s, as a negative control. Lung epithelial cell toxicity was measured using cell permeability and cell density. Results: Exposure of trachea cells to 5µM NA did not result in significant cell death with or without active liver microsomes. At higher doses of NA, extents of WT cell death were similar (60% drop in cell density at 20µM and 90% drop at 80µM) in cultures containing active WT, active *cyp2abfgs-null,* or boiled microsomes. Following pretreatment of epithelial cells with a P450 inhibitor, piperonyl butoxide, WT cells cultured with active WT microsomes were protected from damage (cell density loss) at 20µM, but not at 80µM, NA. *Cyp2abfgs-null* epithelial cells cultured with active WT microsomes were protected from cell death at NA exposures from 5 to 80µM. Conclusions: Airway epithelial cell NA metabolism after 4 weeks of ALI culture can contribute to toxic metabolite generation. NA airway epithelial cell toxicity in vitro occurs independently of the contribution of liver P450 metabolism for WT airways. Circulating metabolites generated by liver metabolism can contribute to mouse airway cell toxicity when local P450s are globally inhibited. Funding: R01 ES020867, P30 ES023513 and T32 HL007013

**TP203 PAHs, OCPs and PCBs in sediments from three catchments in Durban, South Africa**

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Polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) are common, and often ubiquitous environmental pollutants. These compounds enter aquatic ecosystems through surface runoff and atmospheric deposition, amongst other sources, where they settle in sediments. Aquatic organisms exposed to these compounds can accumulate these compounds in lipid tissue, with potential toxic effects that include cancers and developmental, immuno-, and reproductive toxicities. In this study, 54 sediment samples were collected from an industrial region in Durban, a busy port city in South Africa. Concentrations of PAHs, PCBs and various OCPs were analysed in the sediment. Their concentrations were compared to sediment quality guidelines to estimate possible risks to sediment-dwelling organisms exposed to these contaminants. Sediment samples were extracted by pressurised liquid extraction and subjected to gel permeation chromatography as a clean-up procedure. Sulphur was removed by treating the sample with activated copper. Further clean-up was conducted using silica gel solid phase extraction. The compounds were quantified using a high resolution gas chromatograph coupled to a mass spectrophotometer. PAHs were ubiquitous, at concentrations between 36–6 800 ng/g dry mass (dm). Congener ratio diagnosis suggests the PAHs were derived predominantly from pyrogenic sources. OCPs and/or their metabolites were detected at varying frequencies and concentrations. The restricted-use OCP dichlorodiphenyltrichloroethane (DDT) and its metabolites were the most often detected OCP and were at a high concentration in sediment at some sites. PCB concentrations varied widely, ranging from below the detection limit to 110 ng/g dm. Comparing these results to international sediment quality guidelines revealed a low risk to sediment-dwelling organisms in most instances. There were, however, exceptions, where a moderate to high risk was estimated depending on compound and guideline used. The mean sediment quality guideline quotient was used to estimate the risk associated with all compounds as opposed to individual compounds. This revealed that these chemicals were present in sediment at 17% of the sites at a concentration likely to present acute toxicity to sediment-dwelling organisms.

**TP204 Polybrominated diphenyl ethers (PBDEs) from three cities in Colombia and their probabilistically risk assessment via breast milk in infants**

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Polybrominated diphenyl ethers (PBDEs) were used as additives in plastics as fire retardants that are ubiquitous in the environment. PBDEs have been detected in human breast milk from several countries. The concentration of PBDEs in breast milk provides not only indication of the body levels in the mother but also allows to determine the potential exposure in neonates. PBDEs and their hydroxylated metabolites are structural similar to thyroid hormones (THs) because they can alter the transport of THs or interact directly with the thyroid gland. There is a rising concern about PBDEs on potential health impact in human due to increasing concentrations in pregnant women can alter the homeostasis of THs during the first three months. Milk samples were collected from Bogota, Medellin, and Cartagena cities in Colombia, Samples (n=60) were analyzed treating them with 20 ml of 0.5 M NaOH solution mixture of ethanol and water (9:1 v/v) at 60°C for 5 h. Organic phase was extracted with 3 ml of hexane and repeated this step 3 times. Extracts were combined and concentrate to 1 ml by a gentle nitrogen stream. Extract was load onto a florisil cartridge and eluted with 10 ml of dichloromethane/hexane (1:3) and finally concentrated to 100 µl prior to Gas Chromatography-tandem mass spectrometry (GC-MS/MS) analysis. Potential risk and its uncertainty were obtained by modeling with Monte Carlo that newborn children have exposure to PBDEs. For each simulation, 25,000 iterations were chosen. The daily intake (DI) of the contaminants (BDE-28, BDE-47, BDE-99, and BDE-153) in breast milk were calculated using the following equation: DI = C x F x I. Where DI is the intake dose (ng contaminant/Kg bw /day), C is the concentration of the contaminant in milk on a lipid basis (ng/g lipid), F is the fat content in milk (g lipid/g milk) and I is the consumption of milk / day). The overall concentration of daily intake PBDE (mean ± SD) was 1.96 ± 2.21 and ranged from 0.325 to 12.14 ng/g- lipid wt. The average dose to infants were 0.013, 0.004, 0.0014, and 0.001 µg/Kg body weight per day, for sum of PBDEs, BDE-47, BDE-99, and BDE-153, respectively. In the risk analysis calculated, hazard quotations for sum of PBDEs do not show a health concern. Acknowledgment: Thanks to Colciencias and Universidad de Cartagena for supporting the project No. 110759634967.

**TP205 Prediction of metal bioaccumulation in fish from a proposed mine discharge**

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Trophic food web modeling was used to predict metal (copper, zinc, mercury, arsenic, manganese, and nickel) concentrations in fish that people consume and that may be exposed to a proposed submerged mine tailings discharge in a tropical saltwater system. Modeling evaluated the major pathways by which fish could accumulate metals from the proposed discharge. These pathways included benthic organisms, micronekton and zooplankton, forage fish, and bioconcentration from the water column. Site-specific biological, hydrological, and physicochemical information was used to estimate metal exposure concentrations in the water column and sediments and identify aquatic species that could be exposed directly or indirectly to the discharge. Large diurnal vertical movement of plankton and micronekton in the system was an important exposure factor, which was used in conjunction with predicted dispersion plumes of the discharge vertically and horizontally to conservatively estimate exposure concentrations of metals. Results of this study yielded site-specific bioaccumulation and bioconcentration factors of metals for different trophic levels and predicted fish tissue concentrations. Except for mercury, and to a lesser extent arsenic, biomagnification of metals in fish people consume was not predicted, consistent with observed data from different trophic levels at the site. Highest concentrations were observed in micronekton for copper, zinc, and nickel. Zooplankton had the highest concentration for manganese. Trophic transfer factors for fish people consume ranged from 0.01 (nickel) to 0.72 (zinc) based on site data. Trophic tissue data from other active mine sites in the region supported predictions regarding bioaccumulation estimates of metals in fish consumed by people. Based on conservative exposure factors, fish tissue concentrations are predicted to meet food safety standards for all metals except mercury and arsenic. These metals do not consistently meet food safety standards currently (without any mine discharge being present). The proposed discharge is not predicted to increase tissue concentrations for these metals.

**TP206 Risk and Toxicity Assessment of Heavy Metals in Water, Fish and Sediments Samples from Ikun and Ureje Dams in Ekiti State, Nigeria**

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Heavy metals have bio-importance as trace elements but the bio toxic effects of many of them in human biochemistry are of great concern. They are natural trace components of the aquatic environment, but their levels have increased due to domestic, industrial, mining and agricultural activities. Due to their toxicity and bioaccumulation, it is important to analyze for different types of heavy metals in our Dams and rivers. This study was carried out to assess the concentration of these metals with a view to evaluate the level of pollution in samples of water, sediment and fish (*Tilapia zillii*) collected from Ikun and Ureje dams in Ekiti State, Nigeria. The results showed that the concentrations of the selected metals are higher in the sediment sample than water sample and the aquatic organism used (*Tilapia zillii*). However, it was observed that Cadmium and Arsenic were absent in the water sample. The values of other parameters present are acceptable in accordance with WHO standards this suggests that the water might be suitable for consumption in form of portable water. The sediment shows that concentration of Iron, Lead, and Cadmium are higher than the WHO standards. This elevated level of heavy metals might contaminate the overlying water which might lead to bioaccumulation by the aquatic organisms present. There is need for constant monitoring because as more industries are established in Ekiti State, Nigeria, the level of heavy metals in these dams may increase progressively. Ikun and Ureje dams in Ekiti State, Nigeria are major sources of pipe borne water for people in the State, therefore checking the level of the metals might minimize the risk of health of the population that depend on the dam for their water and fish supply.

**TP207 Risk Assessment of Chromium and Arsenic Deposition on Soils and Crops at Industrialized locations of South East, Nigeria**

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This study investigated the human health risk associated with Chromium(Cr) and Arsenic(As) through consumption of food crops; vegetables (Pumpkin leaf, Bitter leaf, water leaf) Tubers (yam, cocoyam, cassava), fruits (orange, star apple, pawpaw) and Nuts (coconut, kolanut, palm kernel nut), harvested from selected industrialized areas located in the South Eastern states of Nigeria. The concentrations of As and Cr were determined using Atomic Absorption spectroscopy (AAS). Mean concentrations of Cr exceeded WHO set limit of 0.05 $mgkg^{-1}$ for all study samples ranging from 1.850.21 to 1315.400.57 in Irete(Imo State), 0.350.21 to 58.900.14 in Ngwo(Enugu), 1.100.14 to 196.530.04 in Osisioma(Abia State), 1.500.01 to13150.00 in Akwu – Uru(Anambra State), 4.550.07 to 294.250.35 in Ishiagu(Ebonyi State) and 0.060.00 to 116.200.00 for umudike (Abia State, control Area for this Study) all in Mg/Kg dry weight. There was no statistically marked difference for As in both study and control areas. The order of abundance of Cr in the areas were in the order Akwu-Uru > Ishiagu > Irete >umudike> Osisioma >Ngwo . The highest bioaccumulation index in this study was recorded in *Talinum triangulare*(9.77E+00), *Manihot esculenta* (7.67E+00) and *Dioscorea alata*(7.26E+00) suggesting that they could be tried out as bioindicators in phytoremediation owing to their observed chromium uptake pattern. The daily intake of chromium in the areas of study were above the established reference dose on 0.003Mg/Kg/day recommended by FAO/WHO and USEPA. The Target Hazard Quotient(THQ) of Cr for most of the test samples were >1 indicating that people in the study areas may experience significant health risks from intake of chromium through food crop consumption. The average carcinogenic risk values obtained for Cr and As exceeded the USEPA prescriptions, thus indicating a lifetime (70 years) probability of contracting cancer suggesting that they be placed for further consideration as chemicals of concern with respect to the assessed locals.

**TP208 Risk assessment of heavy metal contamination by multivariate and Hazard Index analyses of a pipeline vandalised area in Nigeria**

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Petroleum contamination is a significant contributor of elevated level of toxic heavy metals, which are of great concern to human health, due to their non-biodegradable nature. Agaye community has experienced frequent gasoline spills due to pipeline vandalisation, resulting in the contamination of soil and water sources. The concentrations of metals (Cd, Cr, Cu, Mn, Ni, Pb, V and Zn) in groundwater, surface-water and soil were determined from a total of 216 samples acquired bi-monthly for two years by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) to evaluate the impact of oil spills. Multivariate analyses using principal component analysis (PCA) and cluster analysis (CA) were also used to study the interactions between metals and identify the possible sources of contamination. The concentrations of heavy metals in soil and water samples were in decreasing order of Mn > Ni > Zn > Cu > V > Cr > Pb > Cd and Ni > Zn > V > Cu > Mn > Pb > Cr > Cd respectively. Ni concentration ranged from 0.42–8.05 mg $kg^{-1}$ and 0.10-2.85 mg $L^{-1}$ for soil and groundwater respectively. Ni and V were more enhanced ($p < 0.05$) in soil samples. This study showed that there is a significant relationship between elevated levels of Cr, Cu, Ni and Zn and oil spillage, due to petroleum spills and that residents are vulnerable to and at greater risk of non-carcinogenic hazards if they consumed groundwater. Multivariate analyses showed significant anthropogenic intrusions of two diagnostic heavy metals (Ni and V) for petroleum contamination in the soils and water sources.

**TP209 Screening ecological risk assessments of polymers used in cleaning products: Polyquaternium 6 and Polyquaternium 10**

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Polymers are important ingredients in everyday products including cleaning products. The American Cleaning Institute (ACI) recently sponsored an industry-wide survey of polymers used in down-the-drain cleaning products in the United States whose results were used to identify polymer classes for further investigation based on hazard categorization, data availability, and prioritization for ecological risk assessment. Of these polymers, the class of Polyquaterniums were identified for further study, and within this class, Polyquaternium 6 (PQ6; CAS 26062-79-3) and Polyquaternium 10 (PQ10; CAS 68610-92-4) were selected as case studies for screening level ecological risk assessments using the publicly available data identified from the survey. PQ6 and PQ10 have diverse properties and functions as they are used in cleaning products, in addition to other industrial and consumer applications. While privately held data exist, and were likely used along with publicly available data for internal assessments by cleaning product companies, this assessment is limited to publicly available fate and effects data for PQ6 and for PQ10 variants of molecular weight and charge density. The databases searched in Phase 1 of this ACI project were previously discussed and include (but are not limited to) *Scopus*, *Web of Science*, *Environment Complete* and *Toxline* (including *PubMed*), USEPA ECOTOX (2017), the ECHA Information on Chemicals (2018), the Human and Environmental Risk Assessment (HERA) on ingredients of household cleaning products (2018), and the USEPA Safer Choice Chemical Ingredient List (2018). Several acute and chronic aquatic toxicity data are available for a form of PQ6 used in water treatment (Magnafloc 368). Acute EC/LC50s for PQ6 range from 0.03-0.075 mg/L, and chronic endpoints range from 0.0042 – 0.500 mg/L. The data are limited for PQ10 environmental fate and effects. Only acute aquatic effects data are available, with EC50s for PQ10 ranging from 0.4- 1.2 mg/L. Low to moderate wastewater treatment plant (WWTP) removals for PQ6 and PQ10 are estimated ranging from 13-38%. The fate and effects data for PQ6 and PQ10 were reviewed and evaluated for robustness for use in screening level risk assessments. Preliminary findings and recommendations for next steps to refine and improve the risk assessment are shared. Limitations with using the current limited data set for screening assessments are also discussed.

**TP210 The US Forest Service Worksheet Tool: An evaluation of its application in modern risk assessment and methods for maintaining contemporary relevance**

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The U.S. Forest Service Risk Assessment Worksheet Tool (FSWT) is a highly sophisticated Microsoft Suite based application developed to assist in performing risk evaluations. This highly accessible tool uses a macro-enabled Word processor, Excel spreadsheet, and Access database to guide a user through the risk assessment process, and generate risk values from toxicity data, chemical properties, water and off-site soil contamination rates, and built-in exposure assumptions. Risk assessors are presented with the challenge of adapting tools and models to new risk assessment methodologies as methods are updated. Computational tools such as the FSWT can be adapted to accommodate these refinements to risk assessment methodologies. This analysis will highlight the advantages and limitations of the FSWT, and demonstrate strategies to adapt the tool to accommodate updates to exposure assessment methodologies.

**TP211 Profiling of Environmental Contaminants using GC/Q-TOF**

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The investigation of organic micropollutants is an important aspect of assessing environmental quality. The conventional approach to this monitoring involves analyzing a defined number of target compounds by mass spectrometry with the instrument operated in a selected data acquisition mode for targeted analytes. However, there is evidence that such an approach may significantly underestimate the exposure and risk of pollutants, compared to a more comprehensive untargeted screen. Recent advances in mass spectrometry allows an increased scope of analysis, no longer sensitivity or selectivity limited when using high resolution accurate mass instruments operated in full spectrum acquisition mode. Accurate mass information enhances the amount of detail in the information collected and allows for the determination of both targeted and non-targeted components. One of the challenges that this information rich data can present is determining what samples warrant a more detailed investigation. This work will present some of the workflow possibilities for profiling of samples to quickly identify samples of interest, potential targets for further compound identification. Retrospective targeted data mining also becomes possible for researchers when an emerging contaminant is discovered, further increasing the importance of this data when determining the cause of environmental events. Finally, the use of new instrument capabilities such as Low Energy EI, and expanded retention time and accurate libraries will be demonstrated for compound ID.

## Aquatic Toxicology, Ecology and Stress Response – Part 1

**TP212 A comparative study of the toxicity of the insecticide fipronil to aquatic neotropical species**

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Sugarcane cultivation has been highlighted in the Brazilian landscape with excessive application of insecticide fipronil. The aim of the present study was to evaluate the toxicity of Regent® 800 WG (a.i. fipronil) using a test battery of organisms that are native species with common occurrence in Brazilian freshwaters: Cladocera (*Ceriodaphnia silvestrii*), Oligochaeta (*Allonais inaequalis*), Diptera (*Chironomus sancticaroli*), Amphipoda (*Hyalella meinerti* and *Strandesia trispinosa*) and Amphibia (*Leptodactylus fuscus* and *Physalaemus nattereri*). All experiments were carried out under controlled temperature (25±1°C) and photoperiod (12h light:12h dark) and performed in triplicate. The test with *C. sancticaroli* was made with 240 mL test solution, 60 g artificial sediment and six organisms. For *H. meinerti*, 100 mL test solution was used with five organisms. Both tests lasted 96h. For *S. trispinosa* and *A. inaequalis*, the bioassays were carried in 10 mL test solution, with five organisms for 48h (*S. trispinosa*) and six organisms for 96h (*A. inaequalis*). For tadpoles, contained 5L test solution and five organisms for 96h. For cladocerans, consisted in five neonates for 96h. Species sensitivity distributions (SSD) were constructed to compare the acute EC50 values derived for the native species and other invertebrate and fish species. By means of the SSD, we observed that the species *Chironomus dilutes* is the most sensitive (0.03 µg/L), followed by *Americamysis bahia* (0.14 µg/L) and the *Cheumatopsyche brevilineata* (0.15 µg/L). With respect to native species, *H. meinerti* (1.29 µg/L) showed the greatest sensitivity, followed by *C.*

*sancticaroli* (6.19 µg/L), *C. silvestrii* (9.7 µg/L) and *S. trispinosa* (53.24 µg/L). For *A. inaequalis, L. fuscus,* and *P. nattereri*, no effects were noted on the survival even at the highest concentration tested 100 mg /L for the oligochaetes and 800 mg/L for the tadpoles. Therefore, these species were the least sensitive to fipronil, being more resistant than the fish *Danio rerio* (181 µg/L) and *Oncorhynchus mykiss* (246 µg/L). Studies in Brazil recorded concentrations of 0.1 to 26.2 µg/L of fipronil in rivers in the state of Rio Grande do Sul and 4.23 µg/g in sediment of marginal lagoon of the river Mogi-SP. Based on these records, *H. meinerti* and *C. sancticaroli* would be at risk at such an exposure scenario. The present study demonstrated that toxic effects on tropical species are likely to occur at environmentally realistic exposure levels.

**TP213 A Look at Bacteria, Metals, and Polycyclic Aromatic Hydrocarbons in Reach 3 of the Los Angeles River**

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The Los Angeles River (LAR) watershed receives wastewater and runoff from a variety of inputs including stormwater runoff from nearby roadways and industrial/residential areas. The historic and on-going pollution that flows into the LAR has led to its listing on the U.S. Environmental Protection Agency's (EPA) 303d listing of impaired waterbodies. There are currently numerous efforts underway to increase water quality in the LAR in order to support public access to the urban watershed while protecting human health. The current study aimed to identify storm drains that may be contributing to the pollutant load in the LAR by acting as ongoing contaminant sources. Focusing on Reach 3 of the LAR, an area with active recreation, storm drains on the east and west side of the river were selected as a concern if they were observed to experience on-going and daily persistent water flow into the river. For each identified storm drain, water was sampled in April, 2017 (n = 6) and July, 2017 (n = 5) and assessed for bacteria, total and dissolved trace metals, and polycyclic aromatic hydrocarbons (PAH's). Bacteria loads at the different sites often exceeded recreational standards by the greatest representing the most prominent contaminant of concern in the storm drains. Several samples had PAH's, such as Chrysene, Benz[a]Anthracene, and Benzo[a] Pyrene among others, in concentrations that exceeded other regulatory limits. Metals tested for did not exceed the regulatory threshold used for comparison. These sampled sites represent a continual source of contamination to the LAR and future work should be focused on testing the application of best management practices (BMP's) that could alleviate these water quality concerns.

**TP214 A model to estimate photo-enhanced toxicity of petrogenic PAHs to aquatic organisms in Canadian Boreal Lakes**

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The boreal shield is Canada's largest terrestrial ecozone covering more than 1.8 million km$^2$ and containing more than 22% of the country's freshwater resources. While numerous crude oil pipelines cross the boreal zone, additional information is required regarding the fate and behaviour of spilled oil, and its potential to induce toxicity to resident aquatic organisms. In terms of chronic toxicity, polycyclic aromatic hydrocarbons (PAHs) represent the most toxicologically relevant compounds in crude oil. Current understanding of PAH toxicity includes recognition that exposure to UV radiation can potentiate the toxicity of these compounds by up to 100 fold, but models applicable to clear, oligotrophic lakes in the boreal zone are not available. We present a model that describes the potential for enhanced PAH toxicity to freshwater organisms exposed to two commonly transported crude oils (diluted bitumen and conventional heavy crude). The model accounts for ranges of UVA and UVB exposures in lakes with varying dissolved organic carbon (DOC) content, standing phytopklankton biomass and total suspended solids. Coupled with summaries of historic spill volumes, this model specifically addressed the potential for photo-enhanced toxicity of PAHs to different life stages of model freshwater organisms in the boreal shield region.

**TP215 A sulphate and total dissolved solids (TDS) toxicity interaction study for coal mine influenced waters in British Columbia**

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The toxicity of sulphate to many freshwater organisms decreases with increasing water hardness, which is accounted for in a hardness-dependent British Columbia water quality guideline (BC WQG) for sulphate. However, the BC WQG recommends site-specific evaluation for very high hardness conditions due to uncertainty regarding cumulative effects of component ions in the mixture. Hardness in mine-influenced receiving waters of many coal mines in BC is currently or is predicted to increase to very high hardness. We conducted a sulphate and total dissolved solids (TDS) interaction study to address these uncertainties. The overall objective was evaluate whether sulphate, along with related ions in a site-relevant mixture, is of potential concern with respect to toxicity in mine-influenced waters under current and modelled future conditions. A related objective was to assess how the toxicity of sulphate is expected to change as modifying factors such as TDS and hardness increase over time based on predictions of future operations. Site-specific, chronic toxicity tests were conducted following standardized laboratory protocols using *Ceriodaphnia dubia*, *Hyalella azteca*, *Centroptilum trangulifer*,and rainbow trout (*Oncorhynchus mykiss*). Mine-affected water samples were spiked with a series of sulphate concentrations added as calcium and magnesium salts in an ion ratio consistent with site waters. The range of tested sulphate concentrations encompassed current site conditions, predicted increases under future conditions, and concentrations anticipated to yield significant effects. A 25% effect on *H. azteca* growth (560 mg/L) and *C. triangulifer* emergence (840 mg/L) were observed in one test water, whereas effects concentrations were higher in the other test water. Apart from these results, the invertebrate species exhibited no effects on survival and sublethal endpoints at sulphate concentrations up to 1,400 mg/L. Rainbow trout exhibited variable responses, but on average showed no effects on embryo-alevin survival and development at sulphate concentrations up to 1,100 mg/L. For all four species, increasing hardness and associated concentrations of major ions did not appear to increase sulphate toxicity under the conditions tested. This study demonstrated that testing of site-relevant ion mixtures (i.e., calcium and magnesium increasing with sulphate) is more relevant to coal mines than the sodium-sulphate toxicity testing upon which the BC WQG for sulphate is based.

**TP216 A synthesis of mercury and selenium concentrations in freshwater fishes in the Western United States**

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Mercury (Hg) and selenium (Se) have the potential to threaten wildlife and impede ecosystems restoration efforts across a range of terrestrial and aquatic habitats. Hg has a strong binding affinity for Se that could potentially reduce Hg bioavailability, bioaccumulation, and ultimately toxicity, yet the implications of these interactions for mercury monitoring and policies remain unclear. We evaluated paired Hg-Se concentrations for 8,657 freshwater fishes in the Western United States, that represent a range of exposure gradients, tissues, species and habitats. Hg and Se were negatively correlated in linear space, but this pattern is common for many trace elements in the environment because trace element distributions are generally log-normally distributed (right skewed). Therefore, another approach is necessary to interpret Hg-Se patterns. Hence, we constructed statistical models to determine the predominant drivers of Hg concentrations and founds that Se explained only a small proportion (97.5% of samples (as found in previous smaller scale surveys), suggesting that finding a freshwater fish in the Western US with insufficient Se to (supposedly) protect against Hg toxicity would be exceedingly rare. We conclude that a protective role of Se against Hg bioaccumulation is not detectable in freshwater fishes in the Western United States based on their co-occurrence in the environment. Interactions between Se and Hg are complex, dynamic, and their role in Hg or Se toxicity require studies that focus on understanding specific mechanisms and take into consideration ecologically relevant exposure concentrations, chemical species, and toxicological effects.

**TP217 Acute toxicity of ionic liquids with differing chemical structures to *Daphnia magna***

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Ionic liquids (ILs) are an emerging chemical class of importance to industrial activities and biofuel production. Ionic liquids are ionic compounds that are liquid at room temperature. As interest increases to create more sustainable biofuels, much research has been performed to understand the use of ILs to decrease the use of toxic solvents. ILs with methoxylated cations can be prepared from renewable resources (e.g., lignin-derived vanillin) rather than petroleum derivatives that are common for other ILs, which have implications for sustainability. However, as production of ILs increases, it is important to quantify toxicity of these ionic compounds to ensure minimal risk to environmental organisms in the case of a chemical spill or from industrial waste. At the same time, for industrial purposes it would be important to identify the most efficient ILs that are also the most environmentally benign. We present here an investigation into the acute toxicity (48 hour LC50s) of different ILs to *Daphnia magna* in relation to chemical structure of the cation and anion. We investigated four ILs: benzyltrimethyl ammonium hydroxide, benzyltrimethyl ammonium methylsulfonate, dimethoxybenzyltrimethyl ammonium methylsulfonate, trimethoxybenzyltrimethyl ammonium methylsulfonate. We hypothesized that toxicity would decrease with decreasing lipophilicity/increasing polarity of the cation constituent of the IL (in this case, more methoxy groups would be less toxic). The LC50s of all four ILs were greater than 22 mg/L. The most toxic IL was benzyltrimethyl ammonium methylsulfonate (LC50 ± 95% CI = 22.8 mg/L ± 5.0) and it was significantly more toxic than the same cation paired with a hydroxide ion (LC50 ± 95% CI = 97.4 mg/L ± 9.7). Adding methoxy groups to the benzene ring generally decreased toxicity with 2 methoxy groups being the least toxic (LC50 ± 95% CI = 121.3 mg/L ± 31.6) and trimethoxy being intermediate in toxicity (LC50 ± 95% CI = 41.2 mg/L ± 2.4). Our hypothesis was partially supported in that adding methoxy groups decreased toxicity, but not in a linear relationship with increasing methoxy groups. Changing the anion also significantly altered toxicity which was an unexpected result though hydroxide is expected to be a fairly non-toxic anion. Chronic studies should be conducted on those ILs that are deemed of greatest industrial potential to ensure that low levels of ionic liquids released into the environment will not harm aquatic organisms.

**TP218 Acute toxicity of thiocyanate and cyanate to rainbow trout across a range of conditions**

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Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) was observed in effluent associated with cyanide detoxification and acid rock drainage discharges from a gold mine in South America. A review of effluent chemistry indicated that effluent concentrations of nitrogenous compounds (ammonia, thiocyanates, and cyanates) sometimes exceeded published LC50s for rainbow trout. While the toxicity of ammonia and associated exposure and toxicity modifying factors (ETMFs) is well characterized, more limited data exist to characterize the toxicity and ETMF relationships for thiocyanates and cyanates. To address these data gaps, we conducted a laboratory toxicity study of the acute toxicity of thiocyanates and cyanates to rainbow trout across a site-relevant range of temperature, pH, and hardness. Relationships between toxicity and ETMF conditions were evaluated and described with regression analysis. There was no significant effect of temperature, pH, or hardness on thiocyanate toxicity under the conditions tested. Temperature and pH effects were inconsistent and generally small, while the effect of hardness was not monotonic. Cyanate toxicity could not be characterized at low pH of 6.0 due to rapid degradation of the compound under these conditions, which precluded characterization of pH effects in this study. At higher pH of 8.5, where cyanate concentrations were stable, there was no significant effect of temperature or hardness on cyanate toxicity.

**TP219 Adaptation of Leptocheirus plumulosus bioassay to measure bioavailability and bioaccumulation of methylmercury in an oligohaline estuarine environment**

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A bioaccumulation bioassay was developed using the marine/estuarine amphipod *Leptocheirus plumulosus* to assess the effects of fresh and field-aged activated carbon (AC) amendments on the bioavailability and biouptake of methylmercury. The study was performed using highly organic sediments collected from a tidally-influenced oligohaline marsh (~5 ppth salinity) dominated by *Phragmites*. Challenges specifically associated with evaluating methylmercury biouptake in a low-salinity environment required an iterative approach to test method development (trial and error). *Leptocheirus plumulosus* is a surface-deposit feeding benthic invertebrate that tolerates a relatively wide salinity range, and so it is ideal for evaluating biouptake from sediments in a salinity-challenged system. Adverse test conditions encountered in early testing demonstrated the need to carefully monitor redox, pH, DO, etc. and to establish geochemical equilibrium in the test chambers prior to initiating the bioaccumulation phase of the test. So, sediments and water were allowed to equilibrate in test chambers for 7 days prior to adding test organisms, and overlying water quality (pH, oxygen, salinity, ammonia), porewater parameters (metals, carbon, anions, pH and ammonia), redox (Eh), and porewater methylmercury concentrations were monitored during this period. During the 7-day equilibration period, pH and ammonia declined to acceptable levels, and redox stabilized, and sufficient methylmercury was produced. After equilibration, pre-acclimated amphipods were placed in test chambers to initiate a 21-day bioaccumulation phase. Overlying water quality, porewater parameters, redox, and porewater methylmercury levels were monitored on days 7, 14, and 21, and amphipods were retrieved at each 7-day interval to assess methylmercury bioaccumulation. *Leptocheirus* survival and growth was excellent for the duration of the study (in lab controls, field controls, and treatments) and sufficient biomass was available for measuring methylmercury uptake. Challenges encountered and additional modifications to the published *Leptocheirus plumulosus* test method will be discussed and a final test design will be presented.

**TP220 Adjusting Arsenic Aquatic Life Criteria: Toxicity Updates, Arsenic Species, and Species Sensitivities**

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A model has been developed to recalculate fresh and saltwater arsenic water quality criteria using recent toxicological data for arsenic and the specific toxicities of the different species of arsenic typically present within a water sample. The current National Recommended Water Quality Criteria (NRWQC) for arsenic in fresh and saltwater reported in USEPA (1984) and adopted by several state agencies is based on data that is more than 30 years old. A considerable amount of new toxicological data for arsenic is available since the value was initially developed. Additionally, the current recommended water quality criterion for arsenic was derived from data for trivalent arsenic (arsenite; $As^{3+}$), the most toxic of the arsenic species, but is applied to total arsenic. A reevaluation of the NRWQC is provided here that uses recent arsenic toxicity data available from the literature versus the limited dataset from 1984 used in the derivation of the current arsenic NRWQC. Notably, this review supports an alternative, lower acute-chronic ratio. The approach then considers the contribution and toxicities of different arsenic species rather than assuming that As3+ makes up 100% of the water sample at a site. Other arsenic species considered in this recalculation are: inorganic pentavalent arsenic (arsenate or As5+), organic arsenite (org As3+), and organic arsenate (org As5+). Toxicity ratios were developed by comparing As3+ to the other arsenic species. These toxicity ratios were then applied to the adjusted As3+ criterion based on the most recent toxicity data to determine an adjusted water quality criterion for each arsenic species. A weighted average criterion value can then be calculated by multiplying the adjusted water quality criterion by the proportion of each arsenic species averaged across a site. For application to sediment porewater data, an additional adjustment involved evaluating the relative sensitivity of infaunal versus epibenthic and pelagic species to arsenic. These tools are expected to improve the realism of arsenic risk estimates for contaminated sediment sites.

**TP222 An evaluation of two synthetic culture waters dosed with vitamins, selenium, or a combination of the two for optimal culturing of Ceriodaphnia dubia**

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Culturing live organisms for toxicity testing requires optimization of a multitude of variables to maintain healthy organisms over time. Many of these variables center around the quality and chemical composition of the water used to culture the organisms. *Ceriodaphnia dubia* (*C. dubia*) is a sensitive species that can be difficult to maintain in the laboratory. This study was performed in efforts to determine if commonly used culture waters, amended with different supplements that have been previously reported as beneficial, could improve the long-term health of *C. dubia* in our laboratory. This study focused on the effect of two different culture waters containing a vitamin mixture (*i.e.*, biotin, thiamine, and vitamin $B_{12}$) and/or selenium on the health and stability of *C. dubia* cultures. Separate cultures were maintained for 32 weeks using moderately hard mineral water or reconstituted hard water. A series of experiments were performed using the two culture waters with one of the following four manipulations: (1) no manipulation, (2) vitamin addition, (3) selenium addition, and (4) vitamin plus selenium addition, for a total of eight different culture water types. Using survival and reproduction endpoints to assess *C. dubia* health, it was determined that there were no statistically significant differences between seven of the eight manipulations. The only manipulation that demonstrated statistical significance was the reconstituted hard water with selenium which was terminated after 12 weeks due to low reproduction and poor survival. The reproduction in the remaining treatments averaged 32-34 neonates per female in the first three broods and the mean survival of all treatments was >93%. Adding supplements to the two culture waters did not prove to enhance the health of the organisms and only the reconstituted hard water with selenium appeared to have a negative impact on *C. dubia* health. Both un-manipulated culture waters were successful in maintaining healthy *C. dubia* cultures during this study.

**TP223 Analysis of Harmful Algal Blooms in Central Tennessee**

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Harmful algal blooms (HAB's) are destructive hydrological phenomena caused by rapid reproduction of cyanobacteria. These cyanobacteria can potentially release numerous malicious cyanotoxins, such as microcystin and saxitoxin into the water during a HAB event. HAB cyanobacteria grow at alarming rates that can asphyxiate a water body and be harmful to the aquatic life in the surrounding area, as well as pose a threat to any human drinking-water intake facilities located there. The number of HAB's has increased dramatically worldwide in the past few decades and has been linked to the growing number of water bodies experiencing anthropogenic eutrophication and increasing global temperature. According to the Tennessee Department of Environment and Conservation (TDEC), 32 water bodies in Tennessee are susceptible to HAB's based on their water quality history. However, little data exists on HAB's prevalence in Tennessee water bodies, although low levels of microcystin have been found at several agricultural and drinking water intakes around the state. The objective of this study is to seasonally quantify toxin-producing HAB species (e.g. *Microsystis spp* and *Anabaena spp*) as well as toxins that are present at a central Tennessee agriculture-influenced wetland and two drinking water intakes. Water parameters, including temperature, dissolved oxygen, phosphorus and nitrogen levels will be collected at the time of sampling. Toxins will be extracted using a SPE extraction protocol and analyzed via LC/MS. Toxin producing organisms as well as toxin genes present will be identified and quantified using qPCR. Several cyanobacteria genera capable of producing cyanotoxin were found to be present in the wetland during initial summer sampling, including Dolichospermum, Chrysosporum, Aphanizomenon, Anabaena, Oscillatoria, Pseudoanabaena, and Lyngba as well as low levels of microcystin and saxitoxin. The microcystin concentrations ranged from below detection to 0.26 µg/L. The saxitoxin concentrations ranged from below detection to 0.07 µg/L. The peak microcystin concentrations were just below the USEPA's health advisory concentration. Correlations of toxin and organism levels with water quality parameters will also be done. These data show potential risk from cyanotoxins in Tennessee water bodies.

**TP224 Assessing Environmental Surface Water Exposure by Cell Culture-based Metabolomics**

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The presence of anthropogenic contaminants in environmental surface waters often results in potential health risks for humans and wildlife. As promising approaches, 'omics techniques have been developed to assess environmental risk and evaluate potential toxic impacts of chemical contaminants. Metabolomics is the 'omics approach to understanding cell and system level biology, and has been applied in vitro for monitoring biological impacts of water contaminants and aquatic environmental stressors. Combined with high-resolution liquid chromatograph mass spectrometry (HR LC-MS), metabolomics can investigate endogenous metabolites of organisms and cells, and their significant variations under chemical exposure for discovering potential biomarkers, elucidating metabolic pathway alternations, and assessing biological impacts of chemical exposure. In this study, we applied cell culture-based metabolomics to investigate impacted biological pathways by chemical contaminants in surface water samples collected from 8 stream sites. These 8 sites were selected from 38 stream sites collected nationwide with different land occupations and chemical contaminated status, based on the number of detected chemicals in the surface water samples. Two human cell lines (liver cell, HepG2 and brain cell, LN229) and one fish cell line (zebrafish liver cell, ZFL) were exposed to these surface water samples for 48 hr. HR LC-MS was applied to investigate the impacts of chemical contaminants on endogenous polar metabolomes and biological pathways of the cells. The results provide insights into the ecological impacts of environmental contaminants on water ecology risk through a molecular-based evaluation.

**TP225 Assessing sedimentation of the Upper Cache River Watershed, Arkansas through proxy measurements**

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Nearly 50% of all assessed streams in the United States are impaired for not meeting their designated use. The leading cause of impairment is non-point source pollution from agricultural land use. One common practice of streams draining agricultural lands is channelization. Channelization, a form of channel alteration, involves making stream channels deeper and wider to increase the hydraulic loading during flood events. This process can lead to increased stream sedimentation, reduced stream water quality, negatively impacted aquatic biota, and potentially lead to stream impairment. In the Cache River Watershed, located in northeast Arkansas, approximately 70% of the 230-km-long watershed is used for agricultural purposes. Nearly 150 river-kilometers are channelized and over 200 river-km are listed as impaired for not supporting aquatic life with sedimentation from agriculture as the leading cause. The goal of the project is to monitor sediment contributions and water quality of the Cache River Watershed through proxy measurements. Turbidity and total suspended solids (TSS) have been analyzed weekly since October 2017, in 13 spatially independent tributaries in the Upper Cache River Watershed. Preliminary results indicate there is a significant association between TSS and agricultural land use within the subwatershed and, when grouped into levels of agricultural intensity, there is a significant difference in turbidity between low agricultural intensity and subwatersheds classified as moderate or high intensity. In this present study, subwatersheds with moderate and high levels of agricultural intensity exceed the Arkansas Department of Environmental Quality's turbidity limit for channel-altered delta streams ≥25% of the time. These results suggest that the intensity of agricultural activities play an important role in the amount of sedimentation of the Cache River Watershed, which may also be contributing to sediment levels in the Mississippi River and ultimately into the Gulf of Mexico.

**TP226 Assessment of bioaccumulation potential of very hydrophobic compounds in Rainbow Trout and the role of metabolism**

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Given the wide use of hydrophobic siloxane compounds, more care is being taken by legislators around to globe to regulate any potential bioaccumulative and toxic substances in the environment. Likewise, the abundance of the global production of siloxanes demonstrates the importance of these bioaccumulation studies and how best to aid in the addition of the knowledge of how these compounds interact in the environment. Also, with the current guidelines set by government bodies, only one chemical is tested at a time. The present proposal aims to help prove the importance of chemical mixture studies in the hopes of showing the complex nature of a cocktail of chemicals within the environment and any given organism that comes in to contact with said chemicals. The main objective of this study has been to determine in-vivo and in-vitro biotransformation rates of various siloxanes in fish. Concentration-dependent biotransformation will be assessed by reviewing the depuration rate constant over time at varying concentrations to determine if $K_T$ is dependent on the concentration of siloxanes within the organism. Preliminary results have shown varying results between the cyclic and linear siloxanes. Specific plans for these studies are as follows: (1) run individual siloxane exposures to juvenile rainbow trout fish (D4, D5, D6, L3, L4, L5) at a concentration of 4.5mM siloxane/g food for and update duration lasting 21 days. Since the depuration rates for the cyclical siloxanes are very slow, and through previous work done in our lab, this high concentration should suffice to represent a depuration rate value, this will also save time and cost for the project. Also, the depuration phase will be extended out further than that of the linear siloxanes. (2) After analysis of linear samples have been completed, subsequent studies will be run at lower and varying concentration to determine if depletion rates are concentration-dependent. (3) The final in-vivo experiment will be run as a mixture of all 6 siloxane compounds at various concentrations to determine if the presence of multiple compounds will alter the depletion rate of other compounds. Test chemicals for both in-vivo studies include: D4, D5, D6, L3, L4, L5, labeled 13C-L3/L4/L5, 13C-D4,D5,D6 for internal standards, reference chemicals used will include: 1,3,5-trichlorobenzene, 1,2,4,5-tetrachlorobenzene, pentachlorobenzene, hexachlorobenzene, PCB 52, PCB 153, and PCB 209 with pyrene used as an external standard.

**TP227 Cigarette butt litter: A threat hiding in marine sediment**

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Smoked cigarettes get discarded daily on the ground and eventually get transported to aquatic environments by storm water runoff, making cigarette butts a common sight during beach clean-ups worldwide. While the toxicity of many cigarette and tobacco constituents is well known, information is lacking on the impact of cigarette waste on marine sediment. In the current study, cigarette butts were buried into marine sediments in tanks containing seawater, and the exposed sediment was sampled periodically over 60 days. Our aim was to identify toxic chemicals that can leach out from smoked cigarettes and persist in sediment causing a potential negative effect on aquatic organisms. In addition, we aimed to identify some chemical candidates to use as markers of cigarette pollution. Cigarette butts and exposed sediment were solvent extracted and analyzed via gas chromatography/mass spectrometry (GC/MS). Chemicals extracted from sediment were compared to those extracted from cigarette butts to identify compounds present in both. Some tobacco alkaloids and its derivatives (nicotine, cotinine, myosmine, and β-nicotyrine) were the prevalent cigarette constituents being detected at every sampling point. Many linear alkanes were also identified both in cigarette butts and sediment but since they are a common pollutant due to oil spills and boat traffic, the pyridine derivatives were considered the best markers of cigarette pollution within aquatic sediments. Our results suggest that cigarette waste could potentially have both acute and chronic effects on sediment infauna since these toxic chemicals do not just leach out and quickly dilute in the water column but they associate to the sediment for an extended period of time.

**TP228 Consideration of species selection and endpoint design in the construction of Species Sensitivity Distributions**

*M.J. Bradley, Smithers Viscient / Ecotoxicology*

The risk of plant protection products (PPP) to aquatic organisms in Europe can be evaluated through a three tier process. Tier I testing evaluates risk to a small number of standard guideline species, and conservative assessment factors are applied to determine the regulatory acceptable concentration (RAC). If predicted environmental concentrations exceed the RAC, the evaluation of risk must be refined, or use patterns of the PPP must be modified to reduce risk. Tier II testing provides the option of refining risk based on additional laboratory testing, while Tier III testing evaluates risk based on model ecosystem testing. Tier III provides the greatest opportunity for refinement, however, these experiments are quite labor and data intensive, and interpretation may be subjective. Tier II testing largely revolves around standardized testing with potentially non-standard species to better understand taxonomic risk. A species sensitivity distribution (SSD) provides the opportunity to reduce Tier I safety factors from 100 to as little as 3, but its utility depends on the relevance and reliability of the test data collected. In terms of relevance, the test species must be reasonably sensitive and taxonomically germane. In terms of reliability, the derived endpoint(s) must be robust, structured, and should be criteria driven. When considering non-standard species, the direct applicability of an established guideline or test method is unlikely, so how does one evaluate the right candidates for construction of an SSD? This presentation will review the necessary considerations when developing an SSD based on testing experiences and industry response. Logistic concepts such as species selection, endpoint selection, test design and reasonable feasibility will be evaluated, as well as regulatory concerns and opinions of the final product that must be considered as part of the overall design.

**TP229 Determining the no observed effect concentration of atrazine to zooplankton for use in metacommunity studies**

*R. Bowser, A. Dzialowksi, J.B. Belden, Oklahoma State University / Integrative Biology*

The effects of pesticides on aquatic ecosystems are most frequently studied in the framework of single species effects or under the assumption that local communities are isolated. Using metacommunity theory, biodiversity dynamics and spatial patterns of species distributions and abundances can be explained through dispersal and linkages between different spatial scales. The broader objective of this research is to understand how metacommunity dynamics affect zooplankton community responses to stressors that directly affect their resource base (phytoplankton). Before conducting metacommunity studies, however, individual sensitivity tests to the chemical atrazine were conducted to determine the concentration of atrazine that has no or limited direct effects on several species of zooplankton, yet inhibits the growth of green algae. Several species of zooplankton were collected from a local lake and cultured until the third generation. Neonates of each species were exposed to atrazine at 0, 300, and 3,000 µg/L. The neonates were exposed until their second brood (11+ days) for chronic sub-lethal and lethal endpoints of atrazine exposure. *Lathonura rectirostris* experienced 100% mortality at 3,000 µg/L atrazine, 25% mortality at 300 µg/L atrazine, and no mortality in the control. *Ceriodaphnia dubia* had no mortality throughout the trial, but experienced growth inhibition before the first brood in the presence of atrazine. *Ceriodaphnia dubia* individuals within both atrazine treatments also exhibited delayed production of the second brood and produced fewer neonates than the control. These results suggest that these zooplankton taxa will experience direct negative effects of atrazine exposure at 300 µg/L and thus atrazine concentrations need to be lower if the community study seeks to assess only indirect effects resulting from inhibition of green algae.

**TP230 Effect characterization of three naphthalene sulfonates (NSAs) freshwater biota and their environmental concentrations in Ontario river sediments**

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Naphthalene sulfonic acids (NSAs) are used extensively in Canadian industries (e.g., dispersant in dyes, rubbers, pesticides or anti-corrosive agent in coatings, gels, sealants) despite the gap in our knowledge of how they may affect aquatic biota upon their introduction to the environment. This study examined the toxicity of three priority NSA congeners; dinonylnaphthalene disulfonic acid (DNDS), barium dinonylnaphthalene sulfonate (BaDNS), and calcium dinonylnaphthalene sulfonate (CaDNS) to some potentially affected aquatic species. Chronic effect characterization was done by exposing organisms via spiked substrates to emulate relevant environmental exposure routes, as these chemicals have a relatively large affinity for organic carbon (log Koc). The 28-d LC50s for juvenile *Hyalella azteca* in sand were >500, 113, and 69 µg/g dry weight (dw), and in sediment were >2000, 832, and 648 µg/g dw, for DNDS, BaDNS, and CaDNS, respectively. Mortality was not a sensitive indicator of toxicity for *Tubifex tubifex*, so we instead examined the more sensitive endpoint, juvenile production. The 28-d EC50s in sand were 638, < 20, and < 20 µg/g dw, and in sediment were 2336, 398, and 205 µg/g dw, for DNDS, BaDNS, and CaDNS, respectively. All endpoints from every exposure scenario indicated that the NSAs examined were significantly more toxic to aquatic biota when present in substrates of lower organic carbon (e.g., sand); the organic carbon content of the sediment (2% in the current study) appears to have acted as a sink for NSAs and reduced their bioavailability and thus toxicity. Interspecies differences in toxicity may be the result of differences in benthic zone occupancy as well as in diet. To represent watersheds where there may not be high levels of organic carbon, acute water-only exposures were conducted with glochidia of the pocketbook mussel (*Lampsilis cardium*), the adult ramshorn snail (*Planorbella pilsbryi*), and a juvenile amphipod (*H. azteca*). As little is known about the environmental concentrations of NSAs, sediment samples were collected from 15 river sites across southern Ontario, some of which are located downstream of wastewater treatment plants. These results will be discussed in the context of observed toxicity in the laboratory to provide environmental relevance. This study will support the aquatic risk assessment of this group of chemicals in Canada.

**TP231 Effect-based analyses of complex contaminant mixtures present in agricultural water, using *Daphnia magna* and *Pimephales promelas***

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Agricultural runoff often contains dynamic and complex mixtures of pesticides that can affect aquatic organisms. Multiple pesticides are routinely detected in creeks that receive agricultural runoff. Amongst these, two emerging insecticides of concern, Chlorantraniliprole and Imidacloprid, have been associated with high mortalities. These insecticides possess novel modes of action, and their sublethal effects on aquatic organisms (invertebrates and fish) are not well characterized. This study investigates effects of complex chemical mixtures on an aquatic invertebrate and fish species, at environmentally relevant concentrations. Agricultural water samples were collected from sites prioritized by the Department of Pesticide Regulations (DPR) for surface water monitoring. Acute toxicity (96h exposure) tests were conducted on the two test species (*Daphnia magna*and Fathead minnows; *Pimephales promelas*). Expression of genes associated with metabolism, nervous system function, and general stress response were evaluated in both species. Two of six tested sites resulted in 100% *D. magna*mortality. These same sites resulted in approximately 10% mortality to *P. promelas.*Altered gene expression was determined for both species. For *D. magna*, there was up to 0.35-fold downregulation of *catalase*(oxidative stress), and variable responses across sites in SERCA1, involved in muscle function; up to 1.74-fold upregulation in some sites, but downregulated up to 0.4-fold in another site sample. Similarly for *P. promelas*, genes associated with calcium signaling or neurological function (*VGCC /CaV1.1 L-type*) were downregulated up 0.11-fold, while a gene involved in involved in DNA repair (*ogg1*) was upregulated up to 3.1-fold in some site samples. Understanding sublethal effects posed by exposure to emerging contaminants of concern is an often-overlooked component of the environmental toxicology toolkit. Research done using environmentally realistic concentrations of multiple contaminants is necessary to accurately assess deleterious effects in a dynamic environment. Further research is required, however, to elucidate the effect of individual chemicals present in agricultural creeks. Here we present analytical chemistry as well as effect-based data from exposure to ambient water samples impacted by agriculture.

**TP232 Effects of binary mixtures between broad class of antibiotics and the azole fungicide in growth inhibition testing of the green alga *P. subcapitata***

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In most cases, ecotoxicological tests are performed by using individual chemicals. However, since a number of chemicals inevitably coexist in the environment, organisms are exposed to many chemicals simultaneously.

Therefore, tests that use several combined chemicals should be performed to predict effects on actual ecosystems. Currently, antibiotics are detected frequently in the aquatic environment as results of their increased use in humans and animal livestock disease and their incomplete removal by various wastewater treatment processes. The potential environmental impacts of antibiotics must therefore be evaluated similar to those of other chemicals. Generally, antibiotics principally influence the growth of prokaryotes rather than that of eukaryotes. However, macrolides such as erythromycin and clarithromycin have been shown to strongly inhibit eukaryotic algal growth in ecotoxicological studies using the green alga, *Pseudokirchneriella subcapitata*. Azole fungicides, which inhibit the ergosterol biosynthesis pathway, are used widely as both topical and oral pharmaceuticals. As with antibiotics, they are frequently detected in the surface waters and sediments of the aquatic environment. Besides their inhibitory effects on the ergosterol biosynthesis pathway, azoles such as ketoconazole, fluconazole, and miconazole inhibit some species of cytochrome P450s (CYPs), and the interactions between azole fungicides and other drugs metabolized by CYPs have therefore been known well in animals. In algae, the interactions of azole fungicides and other drugs metabolized by CYPs have not been known so far, but it may provide synergism as in animals because algae have been reported to have greater family of CYPs than animals. Here, we investigated effects of binary mixtures between an azole fungicide ketoconazole and broad class of antibiotics (macrolides, lincosamides, tetracyclines, amphenicols and aminoglycosides) on algal growth. Combination index plots, isobolograms, and curve-shift analyses revealed that the combination of ketoconazole and all the selected antibiotics except for neomycin at various ratios resulted in strong synergism that enhanced growth inhibition of *P. subcapitata*, suggesting the existence of antibiotics metabolic pathway by ketoconazole-targeting CYPs in *P. subcapitata*, as existing in animals. However, identification of the species of CYPs targeted by ketoconazole still remains unclear and need to be investigated in the future.

**TP233 Evaluating the combined toxicity of metals and metal-oxide nanoparticles in marine microalgae**

*R.B. Ogunjemilusi, University of Bristol / School of Biological Sciences*

Coastal aquatic ecosystems such as estuaries are at risk of metal pollution due to anthropogenic inputs from acid mine drainage, industrial and agricultural run-off. In addition, advances in nanotechnology in the last decade have increased the production of engineered nanomaterials (ENMs) used widely in fields such as medicine, energy, agriculture, consumer and house-hold goods manufacture. As a result, there are increasing concerns about the release of ENMs such as metal nanoparticles into the environment. While the effect of metals and some ENMs as single contaminants have been extensively studied, much research is needed to account for the potential mixture effects due to heavy metal-ENMs interactions. Studies suggest that the toxicity of metal-oxide nanoparticles is mainly due to the release of dissolved metal ions. However, majority of these studies have mainly focused on impact of ENMs in freshwater environment and results are extrapolated or other types of environmental systems (marine, soil, sediment). Evidence is accumulating that the dissolution of ENMs is dependent on the characteristics of the exposure medium. Metal-oxide nanoparticles tend to form aggregates in seawater medium, reducing the amount of released ions. In this research, the combined toxicity of heavy metals (Cu and Zn) and their metallic oxide nanoparticles (CuO and ZnO) will be evaluated in two marine microalgae – *Cylindrotheca closterium* and *Phaeodactylum tricornutum*. We hypothesize that the aggregates formed by the metal-oxide nanoparticles may adsorb the free metal ions, reducing the bioavailable fraction of heavy metal ions in the exposure medium. Because of intrinsic ecological differences in both microalgae; *C. closterium* having a bottom dwelling nature and *P. tricornutum* mainly planktonic, we also investigate if toxic response may be due to differential mechanism of toxicity. For instance, reduced light intensity as a result of ENMs aggregation may be expected to be more prevalent in benthic microalgae-ENMs exposure than in test systems containing planktonic algae exposed to ENMs.

**TP234 Evaluating the toxicity of California stream sediments with the amphipod *Hyalella azteca*, the midge *Chironomus dilutus***

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As part of the US Geological Survey's National Water-Quality Assessment Program (NAWQA), 81 stream sediment samples representative of urban, agricultural and mixed land use in coastal, central California, were collected in the spring of 2017 to be evaluated under the Regional Stream Quality Assessment project. Relationships between biological conditions and concentrations of contaminants, metals, pesticides, PAHs, nutrients, and sediment in streams from the state of California were evaluated by conducting whole-sediment toxicity tests. The sediments were tested in accordance with ASTM and USEPA methods for conducting sediment toxicity tests with the amphipod *Hyalella azteca* (28-d exposure; 81 samples), and the midge *Chironomus dilutus* (10-d exposure; 40 samples). Toxicity endpoints included survival, weight, and biomass. Mean control survival at the end of the exposures met test acceptability criteria. Sixty-two percent of the sediments (50 of 81 sites) were toxic to at least one of the test species, as determined by a significant reduction of at least one endpoint relative to the control sediment. Amphipod exposures identified 44% (43 of 81 sites) of the sediment samples as toxic. Midge exposures identified 48% (19 of 40 sites) of the sediments as toxic. Results from the 2017 study in California will be compared to the other NAWQA regional sediment-toxicity surveys conducted in the Midwest (2013), the Southeast (2014), the Pacific Northwest (2015) and the Northeast (2016). Five major classes of sediment pollutants were measured in these stream sediments, Organochlorines, PAHs, total PCBs, metals, and current use pesticides. Ongoing efforts will be presented, including development of a reference envelope approach to compare organism response to reference sediments, and comparisons between sediment toxicity and sediment chemistry.

**TP235 Fate and Effects of a Copper-Based Algaecide to Mitigate a Recent Invasion of *Nitellopsis obtusa* (Starry Stonewort) in Lake Sylvia, Minnesota**

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Pulse algaecide exposures are intentional inputs of pesticides into water resources to mitigate algae that impede or threaten to impede uses of the water resource (e.g. recreation). Risks for non-target (i.e. native) algae and macrophytes occur when these organisms co-occur with the deleterious alga(e) targeted by an algaecide exposure. *Nitellopsis obtusa* (Desvaux in Loiseleur) J. Groves (starry stonewort) is a non-indigenous alga in North America that spread rapidly among inland lakes across the Great Lakes region of the U.S. A copper-based algaecide was used in Lake Sylvia (Wright Co. MN) to mitigate potential impacts of a recently established population of *N. obtusa*. To inform risk management decisions for *N. obtusa* in the U.S., overall objectives of this study were to 1) measure and compare responses of *N. obtusa* and co-occurring native macrophytes to algaecide exposures in the area of Lake Sylvia infested with *N. obtusa*, and 2) to compare risks of mitigated *N. obtusa* in Lake Sylvia with an unmitigated population in a nearby water resource (i.e. Lake Koronis, southwestern Stearns County). A chelated copper-based algaecide was applied 4-times between June 21 and October 17, 2017, in a 0.65-hectare area around the boat access in Lake Sylvia, in response to sprouting *N. obtusa* bulbils. Copper concentrations measured immediately after each algaecide application were >90% of the intended initial concentration (1000 µg Cu/L in the bottom 0.61 m of the water column), and post-treatment dissipation followed first order rate kinetics. Half-lives of copper exposures were between 10.9 and 12.8 h. As a result of algaecide exposures, the occurrence of *N. obtusa* in rake samples in the treated area declined significantly from May (21.3% occurrence) to October (0.0%

occurrence). Decreases in the density of *N. obtusa* bulbils (bulbils/m$^2$) in the treated area were also measured. In contrast to Lake Sylvia, occurrence of unmitigated *N. obtusa* in rake samples in Lake Koronis was between 80% and 100% (n=15) during the same time period (i.e. May to October). Although changes in the occurrence of some native macrophyte taxa were measured in Lake Sylvia, the cumulative occurrence of all native macrophyte taxa did not change significantly in the treatment area as a result of algaecide applications. The results of this study indicate that algaecide exposures can mitigate risks associated with *N. obtusa*, while risks for non-target macrophytes and algae are minimal.

**TP236 Heavy Concentrations in Some Fresh Fishes near an Electronic Waste Dumpsite (Agbogbloshie) and a Control Site (Asesewa) in Ghana**

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Background: Processing of electronic waste could result in release of toxic chemicals including heavy metals into the surrounding environment. These toxic substances could end up in the food chain, including vegetables and fish over time. Elevated levels of toxic heavy metals in food could have direct adverse health implications for humans. This study assessed and compared concentrations of Pb, Hg, As and Zn in selected fish species from Korle-Lagoon (main site), near the Agbogbloshie e-waste processing/dumpsite and the Volta Lake (control site). Methods: Concentration of heavy metals in fish except Hg was determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Mercury (Hg) in fish was measured using the Direct Mercury Analyser (DMA). Statistical analysis was done using SPSS version 22 and reported in means and standard deviations. Results: In total, 84 fish tissues (muscle, liver and gill) were analyzed. The *Ameiurus catus* from the Korle-Lagoon hadhigh levels of Pb (0.62±0.65 µg/g dry wt) and Zn (154.21±143.51 µg/g dry wt) whilst the *Hepsetus odoe* from thesame sitehad the highest concentration of As. However, the *Hepsetus odoe* from the Volta Lake had highest level of Hg (1.08±1.22 µg/g dry wt).High concentrations of As and Zn were observed in the three tissues with Pb levels only high in the liver and gills. Between sites, the Korle-Lagoon recorded higher levels of Pb, As and Zn in fishes sampled. High Hg (0.62±0.97 µg/g dry wt) concentrations was found in fish from the Volta Lake. Conclusion: Irrespective of site, the *Ameiurus catus* had high levels of Pb and Zn whilst the *Hepsetus odoe* high levels of As and these were above permissible limits according to the WHO standards.The liver and gills had high levels of As, Pb and Zn. Overall, these fish species were not considered safe for human consumption.

**TP237 Impact of low-concentrated chemical mixtures on zebrafish embryos (*D.rerio*)**

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Recent environmental monitoring studies indicated that aquatic micropollutants occur in multitudes of low-concentrated chemicals. The joint effect of low-concentrated chemical mixtures is discussed as potential cause for low ecosystem quality. Even mixtures containing only chemicals below their individual no observed effect concentration (NOEC) can cause combined adverse effects (principle "something from nothing"). Whether this can be detected or even predicted in complex model organisms, such as the zebrafish embryo (ZFE), was investigated in this study. Therefore we analyzed the impact of low-concentrated chemical mixtures on *D.rerio* embryos and the predictive power of two available mixture toxicity prediction concepts – the model of Concentration Addition (CA) and Independent Action (IA). For this reason, ZFE were exposed to several single substances in various concentrations, hence adverse effects recorded and dose-response curves (DRC) calculated. Subsequently, single substance toxicity data were used to model five individual mixtures. Two mixtures were prepared to investigate the CA model, hence containing similarly acting substances. Another two mixtures were analogously prepared which contained dissimilarly acting components, thus addressing different modes of action and the IA model. Here, an equitoxic design was chosen at which selected chemicals were mixed in their individual $LC_{10}$ values obtained after exposing ZFE for two different treatment periods (0-48hpf and 24-72hpf). To scratch limits at which a remarkable adverse effect induced by low-concentrated chemical mixtures is detectable, a fifth mixture consisting of 13 environmentally relevant chemicals, mixed in their individual NOEC ($LC_0$-$LC_{0.1}$), was investigated. After exposing ZFE to different mixtures, observed mixture toxicity was elucidated and obtained DRC compared to predicted mixture effects. In summary, results revealed a joint mixture effect for all tested mixtures. More precisely, mixture effects appeared always within the prediction window of the CA and IA model. The IA model usually underestimated the mixture effect whereas the CA model had higher predicted power. Altogether, a concerning joint mortality up to 90-100% was reached even if components were applied in NOEC or low-dose concentrations ($LC_0$-$LC_{0.1;}$ $LC_{10}$).

**TP238 Impact of Polystyrene Microsphere Ingestion Indicating Internal Styrene Leaching using *Orconectes virilis* as a Model**

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The ingestion of microplastics is a relatively new ecological and human health concern. Plastic microbeads, which rarely exceed 1.0mm in diameter, are now commonly used in cosmetic products. Due to the inability of many U.S. wastewater treatment plants to remove microbeads, these plastics are released within effluent into local bodies of water. Ingestion and tissue accumulation of microplastics has been identified within a wide variety of aquatic organisms, including decapod crustaceans. However, little is known about the toxic effects of these microscopic synthetic polymers upon ingestion. This study investigated the toxicity of 10mm and 45mm diameter polystyrene microspheres, sizes commonly found in cosmetic products, on *Orconectes virilis*, a Hudson River native crayfish species. Four exposure and three control treatments were established: 10mm polystyrene microspheres in a low and high concentration, 45mm microspheres in a low and high concentration, stationary control, Hudson River water flow through control, and Hudson River cage control. Each glass aquaria or cage contained two male and two female crayfish for a 14-day exposure period before harvesting. Significantly higher levels of DNA damage within the hepatopancreas were seen in exposure groups compared to controls. Slight variations of $Ca^{2+}$ and $Fe^{2+}$ concentrations were seen in both exoskeleton and hepatopancreas tissue between control and exposed crayfish. Differences in response intensity during the experimental period were insignificant, however, 62.5% of exposed crayfish underwent ecdysis compared to 25% of control crayfish. Pathological differences of hepatopancreas and exoskeleton tissue were evident as hepatocyte cells displayed higher levels of vacuolization, cell hypertrophy, and cell membrane degradation in exposure groups, as well as demineralization and undefined layering of the exoskeleton. These results correlate with literature regarding the toxic effects from exposure to the monomer styrene, which can be found within the polystyrene matrix and leached from the polymer in the presence of high temperature, acids, oils, and other compounds. This study's findings suggest that styrene may be leached internally, after polystyrene ingestion. This not only poses a threat to aquatic organisms and ecosystem health, but also to human health as crustaceans are commonly consumed worldwide.

**TP239 Impacts of water quality parameters in an agriculturally dominated watershed: Bayou DeView, Arkansas**

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Bayou DeView Watershed, a tributary of the Cache River located in northeastern Arkansas, provides a variety of important ecosystem services. Watersheds provide ecosystem services including climate and disturbance regulation, water regulation and supply, erosion control, nutrient and waste cycling, and aesthetic and recreational values. Disruption of watershed functioning from impairment largely results from anthropogenic activities. Impairment can result in decreased biodiversity, the creation of hypoxic zones, and ecosystem death. Bayou DeView is characterized as a channel-altered Delta Ecoregion stream. Channelization refers to the deliberate straightening of streams and rivers that usually involves removing meanders, widening, and dredging the stream channel. Channel modification increases total suspended solids, nutrient run-off, and alters diversity of flora and aquatic fauna.Possessing a high diversity of pollutant tolerant aquatic organisms, Bayou DeView offers near permanent surface water habitat and agricultural irrigation supply. Bayou DeView is impaired by excess sedimentation. Seven sites selected within Bayou DeView Watershed were sampled weekly for physiochemical and nutrient water parameters. Analysis of parameters were performed as a function of monitoring site, season, agricultural cropland percentage, and stream order. Physiochemical parameters were significantly different among sites ($p< 0.01$). Seasonality significantly affected temperature and total phosphorus levels ($p=0.02$; $p=0.001$). Agricultural cropland percentage and stream order were not significantly related to any physiochemical water quality parameters ($p>0.05$). Continued consistent monitoring and assessment of surface water in Bayou DeView Watershed is vital in preserving ecosystem services and assist in the implementation of best management practices.

**TP240 Influence of Post Hatching Age on the Sensitivity of Zebrafish (*Danio rerio*) Acute Toxicity Test**

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Acute fish toxicity (AFT) test have been used in many countries for effluent risk assessment and/or ecotoxicological monitoring. Within it, a relevant number of countries have used OECD 203 – or some national standard protocol with minor differences like Brazilian NBR ISO 15088 – as a standard method. This protocol determines the usage of juvenile/adults fish within a specific length size (2 ± 1 cm) that may not be directly associated with organism's age. Some studies have suggested a clear decrease in sensitivity to pollutants along the organism lifetime, resulting in a higher sensitivity of larvae when compared to adults. Summing up, tests with adults require a large volume of tested solution (chemicals, effluent…) when compared to larval tests, resulting in logistic costs. Due to that, the Environmental Protection Agency (USEPA) recommends that toxicity tests should be performed with organisms that are in the early stages of development, ensuring the most sensitive stage to be tested. The objective of this research is to strictly determine the optimal age for the acute fish test using zebrafish (*Danio rerio*) as a model. Fish were reproduced in the laboratory and nurtured with *Paramecium* sp. *Ad libitim* until 2 hours prior testing. Toxicity tests were performed in replicate for organisms aging from 2 to 24 days post-hatching using Potassium Chloride (KCL) as the reference solution. Until the moment, 45 tests were performed. Organisms were exposed to the reference solution for 48 hours in a static renewal system and kept at 25 ± 2 °C. LC50:48h were determined using Trimmed Spearman-Karber 1.5 Software. The sensitivity curve presents a clear increase in zebrafish sensitivity to KCl between the 4th and 14th day of life. Notwithstanding, further studies aiming other reference solutions still need to be concluded.

**TP241 Inhibition Growth Effect of Sunscreen UV Filters on the Freshwater Microalga *Scenedesmus acutus***

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As the use of personal cosmetic care products (PCCPs) with organic ultraviolet (UV) filters are increasing, so is the exposure risk of these compounds to aquatic ecosystems. This study focuses on the inhibition growth effect of 6 common UV filters found in PCCPs on the freshwater microalga, *Scenedesmus acutus.* Fluorescence of chlorophyll was used as a measure of growth during a 96-h exposure period and growth inhibition was utilized as the endpoint. All UV filters inhibited growth with increasing concentration, except for avobenzone, which did not decrease reproduction at any treatment level up to water solubility. Lowest observed effect concentrations were greater than 100 ppb without UV light. Homosalate was the most toxic and avobenzone was the least toxic. Further testing will compare each of the UV filters under UVA and non-UVA conditions to see if UVA radiation increases toxicity.

**TP242 Laboratory Evaluation of Impacts of Exposure Concentration and Duration on Forestry Herbicide Toxicity to Aquatic Plants and Algae**

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The use of herbicides to control competing vegetation during stand establishment is a key component of intensive silviculture. Although some field studies have shown that the use of modern best management practices is effective at reducing the movement of applied herbicides into forest streams, peak stream water concentrations of some herbicides still exceed no observed effects concentrations (NOEC) for some aquatic plants. Exposure duration is a key factor in determining the magnitude of effects of herbicides on aquatic plants. Following forestry herbicide application, herbicide concentrations in forest streams have been shown to "pulse" in response to storm runoff, with peak concentrations persisting for brief periods of time. Since the studies from which NOEC values are derived are typically based on 7- or 14-day continuous exposures, the short-term exposures associated with forestry herbicide may represent a significant mitigating factor in actual toxicity to aquatic plants. We used a hybrid approach to quantify the duration-exposure concentration-toxicity relationship for herbicide mixtures and aquatic plants (*Lemna gibba*; OCSPP 850.4400: Aquatic Plant Toxicity Test Using Lemna spp.) and algae (*Raphidocelis subcapitata*; OCSPP 850.4500: Algal Toxicity), and evaluated recovery from exposure of varying durations. Measured endpoints included growth measured as RFU (*Raphidocelis*), and as frond area, counts and biomass (wet and dry mass; *Lemna*). Selected herbicides and mixture ratios represented those most commonly used based on application area in the southeast and Pacific Northwest regions of the US (Glyphosate, Imazapyr, Metsulfuron, Sulfometuron, Hexazinone), with the highest test concentrations calculated using the US Forest Service Risk Assessment of upper peak concentration (mg/L per application of 1 lb/acre) and the highest application rate used by the industry. We describe species-specific test methods and preliminary findings for both taxa.

**TP243 Light emitting diode can be used for green alga growth inhibition tests**

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Alga growth inhibition tests are commonly performed by using a growth chamber with fluorescent lamp (FL) as a lighting source because the test guidelines require continuous lighting with uniform fluorescent illumination. Recent growth chambers, however, are equipped with a light emitting diode (LED) instead of a FL because of the restriction for mercury-added products by the Minamata Convention adopted in 2013. Although both FL and LED have the photosynthetically effective wavelength range of 400-700 and can provide an adequate light intensity according to the guidelines, qualities of light, such as color, intensity of each wavelength, range of wavelength, and so on, are slightly different between them. We investigated the qualities of light from LED and FL and conduced the growth inhibition tests of five kinds of chemicals in a unicellular green alga *Pseudokirchneriella subcapitata* with LED and FL to confirm whether LED can be used as a substitute for FL. Sodium chloride, cadmium chloride, potassium dichromate, 3,5-dichlorophenol and pentachlorophenol were used for the tests because these chemicals have different modes of actions and are assigned as reference substances in the test guidelines. The results showed no difference in the growth inhibition rates and yield inhibition rates between the LED and FL conditions. Moreover, ErCx, EyCx and NOEC values of each chemical under the LED condition showed similar values to the FL conditions. In conclusion, LED instead of FL as a lighting source did not affect the results of the growth inhibition tests with the green alga. Therefore the test guidelines may need to be revised to allow the use of LED though we need to investigate the effects of LED for the other algae, cyanobacteria, diatoms, and marine algae.

**TP244 Long-term ecological effects on infauna communities of wastewater effluent from wastewater treatment processes in Southern California**

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In the last 16 years, the Orange County Sanitation District (OCSD) commenced effluent disinfection by chlorination in 2002 and full secondary treatment in 2012 to improve the water quality of effluent, and partnered with the Orange County Water District on the Ground Water Replenishment System (GWRS) water reclamation project in 2008 to mitigate the water resource crisis in California. To assess the ecological impacts of these processes, the infauna community was monitored off the coast of Huntington Beach, California, from 1991 to 2016. Results showed the Infaunal Trophic Index (ITI) and Benthic Response Index (BRI) at the monitoring station near the OCSD outfall were stable between "normal community" and "changed community" levels from 1991 to 2001. However, after chlorination began in 2002 the infauna community near the outfall became degraded, with ITI falling sharply after 2008 to the lowest value in 2010. However, when full secondary treatment was achieved in 2012, in combination with a substantial reduction of chlorine use in the treatment process, the infauna community quickly recovered to pre-chlorination levels. In contrast to the outfall site, the reference location remained unchanged throughout this duration. Changes of both ITI and BRI near the OCSD outfall before 2012 were correlated with chlorine usage and a decrease in final effluent flow rate, which were not observed at the reference site. These results indicated that disinfection by chlorination and the GWRS were harmful to infauna communities, with associations to chlorine use low-flow periods.

**TP245 Monitoring Water Quality in Eight Subwatersheds of the Strawberry River**

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The Strawberry River is a tributary of the Black River within the White River basin in northernArkansas. The river distance from source to mouth is about 177 km, and it is considered by the ADEQ as an extraordinary resource and ecologically sensitive waterway. In order to protect the water quality, it is important to determine which tributary is adding to the impairment due to siltation. Water quality in eight tributaries (Piney Fork, Mill Creek South Big Creek, North Big Creek, Clayton Creek, East Cooper Creek, Reeds Creek, Sleep Bank Creek, and Caney Creek) are collected prior to the confluence with the Strawberry River. Water quality variables include total suspended solids (TSS), turbidity, pH, conductivity, dissolved oxygen, PO4, TN TP, NO2-, and NO3. Turbidity values range from 0.76 to 64.8 Nephelometric Turbidity Unit (NTU) and TSS values range from 1.6 to 73.6 mg/L. This ongoing project will help understand the water quality of the Strawberry River and the eight subwatersheds analyzed. Additional data for this project include assessment of fish communities in the Strawberry River. The river supports over 100 species of fish, and protection of fish and other indigenous species is important to understand how the water quality impacts the species and their habitats.

**TP246 Occurrence of pharmaceuticals at high concentrations in Nigerian rivers**

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Pharmaceutical contamination of surface waters is increasingly recognized as a global problem, but to date there remains a scarcity of data from many African countries. The occurrence of 37 pharmaceuticals belonging to 19 therapeutic classes was studied in surface water and effluent in Lagos State, Southwest Nigeria. Samples were collected year-round from 22 sites, and 27 compounds were detected at least once, many in the microgram per litre range. Maximum concentrations for a range of compounds including sulfamethoxazole, paracetamol, cimetidine, fexofenadine, carbamazepine, metformin and diazepam ranged from 75031 ng $L^{-1}$ to 129474 ng $L^{-1}$ in areas receiving untreated sewage and pharmaceutical manufacturing plant discharges. Mean concentrations for 13 compounds were in the mg $L^{-1}$ order. These values are several orders of magnitude higher than most studies of pharmaceutical occurrence in Europe and North America but similar to some other peak concentrations measured in developing countries such as China and India. Multiple pharmaceutical compounds were found at all monitoring sites although there were few clear spatial patterns. This may indicate that a variety of pharmaceutical sources exist throughout the catchment, including manufacturing facilities, sewage treatment plants, vacuum trucks that discharge sewage to rivers and urban areas where sewage treatment is not provided. AbSeasonal variations in concentrations were observed for certain pharmaceuticals such as fexofenadine, carbamazepine, paracetamol, cimetidine, metformin, sulfamethoxazole, atenolol, diazepam, codeine, and trimethoprim which may be related to the environmental conditions that affect dilution and degradation and/or seasonal manufacturing and usage. Pharmaceuticals are indispensable to human health although their usage and discharge into the aquatic environment may lead to ecological problems and antibiotic resistance. African governments may need to enact policies to remedy losses to rivers urgently.

**TP247 Oxidative stress produced by aluminum nanoparticles in liver and blood of *Cyprinus carpio***

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The use of nanoparticles in various industries has experienced an important growth due to the advantages they offer, so the increase in their use has generated the continuous discharge of these products in numerous water bodies, and may affect the organisms that inhabit them. Aluminum (Al) is one of the chemical elements that is abundantly produced in particles of nanometric size, having an important application in the pharmaceutical industry. Previous studies have shown that Al is capable of producing oxidative stress in mammals and aquatic organisms, however, so far there are no studies on the impact of Al nanoparticles on hydrobionts, such as *Cyprinus carpio*, a species widely distributed and of great ecological, commercial and food importance in Mexico. Therefore, the objective of this work was to determine the oxidative stress produced by

Al nanoparticles in liver and blood of *C. carpio*. For this, the organisms were exposed to the xenobiotic at a concentration of 50 μg/L for 12, 24, 48, 72 and 96 h, with n=4 for triplicate. Subsequently, 0.5 mL of blood sample was obtained by puncture of the caudal vein and euthanasia of the organisms for the extraction of liver. The determination of oxidative stress was performed by evaluating the degree of lipoperoxidation, the content of hydroperoxides and oxidized proteins, as well as the activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). The results showed alterations in the activity of the antioxidant enzymes as well as damages in lipids and proteins in both organs.

### TP248 Platinum group element exposure to macro-invertebrates from a river impacted by platinum mine effluent

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South Africa is the world's main supplier of platinum group elements (PGE), which include platinum, palladium and rhodium. The Bushveld Igneous Complex in South Africa contains 75, 52 and 82 % of the world's platinum, palladium and rhodium resources, respectively. The mining of these precious metals requires large volumes of water for production and the removal of waste products. Most of this waste water is discharged into river systems, which can have a detrimental effect on the aquatic ecosystem. Although the input of PGE by mining activities to aquatic systems are known, little to no information is available on the influence of the PGE input on aquatic taxa in these systems. However, the uptake and bioaccumulation of PGE by aquatic animals has been clearly demonstrated in laboratory investigations, as well as field studies from non-mining areas. The aim of this study was to determine the bioaccumulation of metals associated with PGE mining in different macro-invertebrate families. Water, sediment and macro-invertebrate samples were collected from a reference, moderately impacted and highly impacted site. The macro-invertebrates and sediment were prepared and concentrations of Pt, Pd, Cr, Co, Cu and Ni in water, sediment and macro-invertebrates were determined with inductively coupled plasma mass spectrometry analysis (ICP-MS). The macro-invertebrates accumulated metals associated with PGE mining and with benthic and epi-benthic dwelling taxa (Tubificidae) accumulating higher Pt, Pd and Cr concentrations than other families (e.g. Potamonautidae, Coenagrionidae and Lymnaeidae). These results provide valuable information on the exposure of PGE mining effluent in the aquatic ecosystems and therefore the risk assessment of these intensive mining activities.

### TP249 Presence of Fecal Bacteria and Associated Pathogens at Recreational Freshwater Beaches in Tennessee, USA

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Fecal indicator bacteria (FIB) have been found in high quantities in the sand of marine and freshwater beaches. Tennessee has more than 30 freshwater lakes and reservoirs, many containing human-made, sandy beaches for recreational use. Although fecal bacteria and associated pathogens in fresh-water beach sand pose a potential risk to the visitors at these recreational areas, as of now there are no federal guidelines for the regulation of fecal bacteria in sand at freshwater beaches. The objective of this study is to assess the abundance of fecal indicators (*E. coli*, coliphages, *Bacteroidales*) and associated pathogens (Methicillin Resistant *Staphylococcus aureus*, MRSA) in freshwater beach sand and water at two central Tennessee recreational beaches. Six sampling events will occur during the summer recreational season in 2018. Three of these events are on major, national holidays which coincide with increased human activity at the beaches. On each sampling date, sand core samples were taken to a depth of ~10 cm at 5 locations in the swash zone at each site using sterile PVC pipe, and water samples were taken at 3 locations in 0.5M deep water using sterile 1L plastic bottles. Sand samples (100g aliquots) were shaken in 1L of phosphate buffered saline (PBS) solution to release bacteria from sand particles before processing. For both water and PBS eluents from sand samples, DNA was extracted using DNeasyâ PowerWaterâ Kits and stored at -80°C until processing by qPCR. Concentrations of *E. coli* in water and sand sample eluents were measured using ColilertÒ, coliphages were enumerated using EPA Method 1602, human-specific *Bacteroidales* were measured using a qPCR assay, and MRSA concentrations were determined with selective agar plates and confirmed by qPCR. Initial sampling results show every beach sand and water sample to contain *E. coli*. The *E. coli* concentrations ranged from 20 MPN-1,989 MPN/100g for swash zone sand, but most sand samples contained less than 400 MPN/100g. Water *E. coli* concentrations ranged from 18.5 MPN/100mL to 90.8 MPN/100g. MRSA was found in all sand samples with concentrations from approximately 120 CFU/100g to too numerous to count (TNTC) and in water from 56 CFU/100mL to TNTC. Ongoing qPCR will be used to verify the identity of MRSA on selective agars. These results show the presence of fecal bacteria and pathogens at these two freshwater sandy beaches and demonstrate a potential risk to beachgoers.

### TP250 Quantifying gemfibrozil disinfection byproducts in southern California sediments and using transcriptomics to assess biological effects

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Disinfection byproducts (DBPs) are a class of compounds produced from wastewater chemical disinfection. Due to the ubiquitous distribution of DBPs in aquatic environments and water supply systems, there is concern for potential health risks in humans and aquatic organisms. Only 18 DBPs are regulated globally and biological effects of DBPs have been minimally studied, especially those formed from pharmaceutical agents. Chlorination and bromination of the anti-cholesterol pharmaceutical gemfibrozil has been observed in wastewater effluent following chlorination and may pose ecological concerns following halogenation. To better understand occurrence and effects of gemfibrozil DBPs, concentrations of chlorogemfibrozil and bromogemfibrozil in Southern California sediments were quantified and toxicity to aquatic organisms was determined through whole-transcriptome sequencing. Sediment cores were collected at the outfall of Orange County Sanitation District (OCSD) where chlorination began in 2002. Following QuEChERS extraction, concentrations of chlorogemfibrozil and bromogemfibrozil were quantified in sediment extracts. To establish toxicity to aquatic organisms, dose-response experiments were performed for gemfibrozil, chlorogemfibrozil, and bromogemfibrozil using *Neanthes arenaceodentata* through aqueous exposures. *Neanthes* were exposed to chlorogemfibrozil and bromogemfibrozil at concentrations found in sediments for 7 days and whole-transcriptome sequencing was used to characterize toxicological effects of gemfibrozil DBPs. Dose-response results in *Neanthes* with gemfibrozil, chlorogemfibrozil, and bromogemfibrozil yielded LC50 values of 469.85, 46.14, and 17.98 mg/L, respectively. Additionally, preliminary data show 0.01 ppb chlorogemfibrozil and 0.03 ppb bromogemfibrozil in OCSD sediments. The results of this research will examine the occurrence of gemfibrozil, chlorogemfibrozil, and bromogemfibrozil in Southern California sediments and quantify the uptake and biological effects of these DBPs in aquatic organisms.

**TP251 Reproductive health of yellow perch *Perca flavescens* populations in Chesapeake Bay tributaries with varying amounts of development**

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Yellow perch have historically been an economically and culturally important fishery to the Chesapeake Bay. Populations, however, have experienced precipitous declines over the past several decades in many regions of the Bay. These declines correlate inversely with percent imperviousness within catchment areas and therefore appear related to human development in the affected watersheds. Females from affected regions are reported to have a high frequency of gamete abnormalities (e.g., thin and irregular zona pellucida (egg envelope), abnormal yolks, and incomplete development at the time of spawn. Additionally, hatching success from impacted populations has declined markedly from > 80% in 1960 to < 10% between 2001 and 2005. Important stages of reproductive development including follicugenesis and vitellogenesis have not been studied in this species. To that end, we conducted an investigation into the reproductive health of mature male and female yellow perch from several historically important spawning reaches within the Chesapeake Bay watershed. The investigation included areas of varying land use from predominantly agricultural (Choptank River) to moderately (Mattawoman Creek) and substantially (Severn River) suburban/urban. We investigated gonadal histology/histopathology, as well as a battery of molecular biomarkers including plasma vitellogenin, circulating sex steroids (e.g., E2 and T), hormones essential to ovarian maturation (e.g., FSH and LH), gene expression within liver and gonads. Yellow perch were collected at several month intervals prior to and on the spawn from each system to investigate gene expression, molecular signaling, hormone levels, and tissue conditions during the period of reproductive recrudescence. The primary objective of the study was to determine the stage and manner that populations from impacted regions begin to deviate from normal reproductive development. Preliminary data will be presented.

**TP252 Sensitivity of California Freshwater Unionid Mussels to Ammonia**

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The inclusion of new toxicity data for freshwater unionid mussels in USEPA's 2013 recommended aquatic life criteria for ammonia resulted in substantially reduced revised national recommended criteria for ammonia; however, the mussel genera included in USEPA's dataset are not resident to the western United States. Species of unionid mussels (*Anodonta*, *Margaritifera*, and *Gonidea* genera) are widely distributed throughout the western United States, yet little is known as to the comparative sensitivity of these resident mussels to ammonia. In order to explore this question, toxicity testing of juvenile *Gonidea angulata* was conducted. Ammonia sensitivity of *Anodonta californiensis* and *Gonidea angulata*is compared to that of other resident species, including *Anodonta oregonensis* and *Margaritifera falcata,* as well to those species represented in USEPA's national dataset for ammonia.

**TP253 Simulation model to study percolation toxicity of pesticides, isolated and in mixtures, in *Raphidocelis subcapitata***

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Bom Repouso (MG) is a municipality with agricultural characteristics and the production of potatoes and strawberry is relevant to the local economy. Nevertheless, in these crops farmers are using the acaricide/insecticide Kraft® (abamectin) and the herbicide Score® (difenoconazole), isolate or in mixture, and the ecological effects are not evaluated yet. Considering that, this research was to assess the effects of these pesticides on the Crustacea *Ceriodaphnia silvestrii,* using studies in aquatic ecosystem models. The experiments were conducted in mesocosms (400 L) containing water, sediment and biological community, simulating the environmental conditions. The pesticides (Kraft and Score) were applied in soil without vegetation, according to the dose recommended by the manufacturer for strawberry crops hich was simulated a rain of 19mm. The runoff of water containing all material transported (control and contaminated areas) was collected and placed in mesocosms, simulating a natural condition of contamination, and then water samples were collected for the acute toxicity tests (48h; n=5), under laboratory conditions, according to the NBR 13373 standards for the species *C. silvestrii.* The results were analyzed by analysis of variance (ANOVA) followed by Dunnett's test at 5% significance level. %. The values of physical and chemical variables were similar in all treatments. However, significant differences were observed for turbidity, in this case the minimum and maximum values were 183 and 380 NTU, respectively, indicating that there is a physical effect, which reduces to 53% and 87% after 24 and 72 hours of application runoff. Regarding toxicity, no significant difference were observed between runoff control, contamination isolated and mixture. This effect can be explained by the short half-life (up to 24 hours in water) of this pesticide, as well as by the adsorption thereof to the particulate material, which promotes its non-availability, reducing the toxic effects on the biological community as mentioned by Novelli et al. (2012).

**TP254 Spatial and Temporal Concentrations of the Neonicotinoid Pesticide, Imidacloprid, in Forester Creek in Santee, CA**

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Imidacloprid, a nicotine-based systemic insecticide, is one of the highest-selling pesticides globally. It is extensively used in both agricultural and urban settings. Neonic insecticides have been most prominently linked to colony collapse disorder in honeybees and pose a significant threat to aquatic invertebrates. The USEPA established benchmarks for freshwater species exposure to imidacloprid include a value of 385 ng/L for acute exposures and 10 ng/L for chronic exposures. Within the United States, concentrations of imidacloprid detected in streams and rivers routinely exceed acute and chronic toxicity endpoints derived for freshwater invertebrates with positive correlation between percentage of urbanization and detection of imidacloprid. Forester Creek, a 16-km tributary to the San Diego River, was the study site of interest. It was chosen due to its previous wetland restoration project and its state of impairment for a variety of pollutants. Imidacloprid was detected with 100% frequency in surface water samples collected from Forester Creek with a mean concentration of 29.56 ng/L (range: 3.77 to 96.83 ng/L). Over 60% of individual samples exceeded the USEPA's chronic exposure benchmark. Temporal analysis displayed significantly ($p < 0.05$) higher levels during wet weather events (47.26 ng/L) than dry weather events (17.76 ng/L), depicting the influence of urban runoff on stream quality. Imidacloprid seemed to follow a first flush pattern with the highest levels observed on the rising portion of the hydrograph as compared to the remainder of the storm again indicating that the build-up and wash off from land surfaces during storms is a major source of this insecticide. The restored wetland/riparian section of Forester Creek did not demonstrate any significant removal of imidacloprid. The occurrence and level of imidacloprid in this urban stream contribute to the limited knowledge on imidacloprid in urban environments and will promote a better understanding of sources and effects of the neonicotinoid pesticide within the urban southern California region.

**TP255 Spatio-temporal variations of zooplankton fauna of Oluwa River, Ilaje Local Government Area, Ondo State, Nigeria**

*F.R. Olaniyan, Adeyemi College of Education Ondo / Biology; A.O. Ugwumba, University of Ibadan / Zoology; A.A. Ayoade, University of Ibadan*

The study undated the knowledge on the status of zooplankton composition of Oluwa River, Ilaje Local Government Area, Ondo State, Nigeria, as indicators of the health of the aquatic ecosystem. The major objective of the study was to provide baseline information on aspects of the biology (taxonomic composition, occurrence, distribution and abundance) of the zooplankton of Oluwa River, Ilaje Local Government Area. Zooplankton samples were collected from six sampling stations with 55µm mesh size plankton net, samples were identified and counted microscopically for eighteen months (June, 2014 – November, 2015) covering both the rainy and dry seasons. A total of thirty-eight species belonging to three groups; Rotifera (30), Copepoda(4) and Cladocera(4) were identified. Rotifera dominated the zooplankton groups, accounting for 87%. *Asplanchna brightwelli* (3.65%) was the most abundant, followed by *A. girodideguerne* (3.47%). The dry season abundance (3373 Org/L) was higher than the rainy season (3025 Org/L) Copepoda accounted for 2% of the percentage abundance of zooplankton. *Eucyclops macrurus* (3.64%) dominated; followed by *Pseudocalanus elongatus* with 3.57% in the relative abundance the rainy season abundance (475 Org/L) was higher than dry season (311 Org/L). Cladocera accounted for 11% of the percentage abundance of zooplankton. *Podon leucarti* (3.64%) dominated, followed by *Ceriodaphinia cornuta* (3.57%) in the relative abundance. The dry season abundance (489 Org/L) was higher than rainy season (476 Org/L). In conclusion, Oluwa River can be inferred to be rich in zooplankton fauna composition and therefore fairly clean and unpolluted.

**TP256 Studying elemental release of engineered nanoparticles used in outdoor painted surfaces with a focus on exposure and toxicity in aquatic models**

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Engineered nanoparticles (ENPs) are used in the manufacture of industrial and consumer products to enable or enhance specific functions towards delivery of high performance products. ENPs in outdoor painted surfaces may be released to aquatic systems with precipitation and other weathering actions though little is known about the fate, exposure and toxicity of released chemicals. The goal of this project is to use two aquatic test models (a – the fish embryo test, FET, OECD TG 236; and b – zebrafish derived cell line, ATCC CRL-2643) to characterize uptake of select elements (focus initially on titanium but also silver and silicon) from painted surfaces across temporal (0-14 days) and treatment (e.g., temperature, humidity, abrasion) gradients. A suite of outcomes will be measured in the cells (cytotoxicity, gene expression, enzyme activity) as well as in the embryos (embryo coagulation, lack of somite formation, tail non-detachment from yolk, and after 48 h the presence or absence of a heartbeat). This work is expected to increase our understanding of potential risks to aquatic organisms associated with ENPs from painted surfaces in outdoor systems.

**TP257 The fate of zinc oxide nanoparticles in aquatic mesocosms**

*S. Bae, Korea Institute of Toxicology; M. Jang, Y. Hwang, Korea Institute of Toxicology / Future Environmental Research Center*

Zinc oxide nanoparticles (ZnO NPs) are extensively used in a variety of products including sunscreens, cosmetics, plastics and ceramics, making their release into environments unavoidable, but their fate in natural environment is poorly understood. In this study, the aquatic systems were made up from natural water and sediment to mimic pond ecosystems and the fate of ZnO NPs were examined in aquatic organisms (*Semisulcospira libertine*, *Oryzias latipes* and *Hydrilla verticillata*). The mesocosms were exposed to Zn at 0.5 mg/L for 50 days. As a comparison, the same concentration of $Zn^+$ (based $ZnCl_2$) were also determined. The results showed that the concentration of $Zn^+$ and ZnO NPs quickly decreased in the water column. The aquatic plant (*Hydrilla verticillata*) and fish (*Oryzias latipes*) significantly bioaccumulated Zn. The results of $Zn^+$- and ZnO NPs- treatment were not different because the ZnO NPs at low concentration were mostly dissolved. Therefore further work is necessary to demonstrate the mesocosms study in the high concentration of ZnO NPs and dissolved Zn ion from particles.

**TP258 Threespine Stickleback as Biomonitoring Tool for Central Oregon's Iconic Rivers**

*D. Heil, A. Petersen, Oregon State University, Cascades*

Central Oregon's famous fly fisheries on the Crooked and Deschutes Rivers are at risk due to river flow draw down for agriculture: agricultural runoff, failing septic systems, and increased human population and growth. Half of the basin that runs along the Crooked River is private land used for agriculture and grazing cattle. Particularly at risk are the native trout and whitefish populations. A resource-efficient method of monitoring the health of these species is to continuously monitor the health of fish that are abundant, easy to catch, not threatened, and live as a resident in proximity to native trout and whitefish. The threespine stickleback (Gasterosteus aculeatus) is an internationally recognized model organism for ecotoxicology which resides in these rivers. We are using threespine stickleback as a wild sentinel species, by looking at pathohisotological, endorinological, ELISA, and molecular approaches to identify impacts to fish health based on location and/or river mile. Stickleback from the Deschutes River exhibit adipocyte density in the liver to be high at three of our seven sampling sites, particularly in male fish. Genotypic male fish at these sites are also producing a large amount of vitellogenin, an egg yolk protein, typically only found in females. We are currently collecting data to map the distribution and abundance of stickleback, pathohistology for disease in fish organs and cells, clutch size and embryo survival for fecundity, gas bubble disease, parasite load, at potentially impacted versus relatively pristine sites. We will discuss these data and their implications to human health and the future of the fishery in Central Oregon.

**TP259 Toxicity of Effluents with Elevated Alkalinity and Conductivity to *Pseudokirchneriella subcapitata*, *Ceriodaphnia dubia*, and *Pimephales promelas***

*N. Lynch, R. Ogle, Pacific EcoRisk*

A Discharger operating under a National Pollutant Discharge Elimination System (NPDES) permit consistently produces effluent with elevated alkalinity (>2000 mg/L) and conductivity (>8000 µS/cm) which are outside of the tolerance limits for freshwater species. The Discharger previously demonstrated effluent toxicity to the freshwater species *Pseudokirchneriella subcapitata*and *Ceriodaphnia dubia*. A third species, *Pimephales promelas*, was tested although no toxicity was observed. It was hypothesized that either the elevated alkalinity or the combination of elevated alkalinity and conductivity were the proximate causes of the observed toxicity. Several tests were performed with each of these three species to test this hypothesis, including: effluent, lab water adjusted to effluent alkalinity ('alkalinity'), and lab water adjusted to effluent alkalinity and conductivity ('alkalinity + conductivity'). The elevated alkalinity of the effluent was mimicked by adjusting synthetic lab water for each of the three species to the effluent alkalinity through the addition of sodium bicarbonate salt. The elevated conductivity of the effluent was mimicked by adjusting the synthetic lab water by the addition of 1µm filtered seawater. Dilution series tests were performed for each round of tests for each species in order produce comparative results. A single test was performed with each of the euryhaline species *Thalassiosira pseudonana* and *Hyalella azteca*, to evaluate the effectiveness of testing alternative species with a greater tolerance range for these elevated water quality parameters. Significant reductions were observed in all tests of *P. subcapitata*and *C. dubia*. However, the magnitude of toxicity decreased in the 'alkalinity + conductivity' test of *P. subcapitata*and decreased in the 'alkalinity' test of *C. dubia*. Toxicity was observed in the 'alkalinity' test of *P. promelas*only. Toxicity was also observed in both of the euryhaline species, indicating that these species would not be suitable as an alternate test species. These results suggest that elevated alkalinity and conductivity play different roles in the toxicity of each of these species, although the toxicity of this effluent cannot solely be explained by these elevated water quality parameters alone.

**TP260 Toxicological effects of tire wear particles on fathead minnow and Atlantic killifish**

*S. LaPlaca, Clemson University / Environmental Toxicology; P. van den Hurk, Clemson University / Biological Sciences*

Recent studies on the distribution of microplastics in the Charleston Harbor, SC, revealed that a large part of the microplastic particles that are found in the intertidal sediments are tire wear particles. These particles originate from the wear of tire treads on roadways, and wash into the estuary during rain events. The abundance of these particles has raised questions about potential toxicity to aquatic organisms that may ingest these particles. The synthetic rubber in car tires consist of a large variety of chemicals, which can be different between brands, but usually contains styrene-butadiene rubber, carbon black and zinc. To investigate the potential toxicity of tire wear particles, both fathead minnow and Atlantic killifish were exposed to different concentrations of tire crumb particles (63-150 um) in a 7 day exposure. Dissection of the fish revealed that particles were actually ingested and accumulated in the intestinal tract. At the highest concentration tested (6000 mg/l) we observed partial mortality in the fathead minnow, which is therefore close to the LC50. To investigate if polynuclear aromatic hydrocarbons were leaching from the particles, bile fluorescence was measured, together with potential induction of cytochrome P450-1A through the EROD assay. In addition, glutathione S-transferase was measured as a general stress parameter. Results will be discussed as comparison between the two species, with the consideration that Atlantic killifish is an estuarine species that is much more used to being exposed to high particle load in its natural environment than the fathead minnow. The results of these experiments can be used to support environmental management strategies that reduce particulate matter in road runoff from entering estuarine environments.

**TP261 USEPA's 1985 Guidelines for Deriving Aquatic Life Criteria – Update on the Status of the Guidelines Revision Process**

*M. Elias, C. Bergeron, USE EPA / Office of Water; L. Cruz, Health and Ecological Criteria Division; A. Jarvis, USEPA / Office of Water; J. Justice, USEPA; K. Gallagher, USEPA / Office of Water*

USEPA's Office of Water is in the process of revising its 1985 *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (Stephan et al. 1985). The Guidelines establishes methods for the derivation of national Aquatic Life Ambient Water Quality Criteria, under Section 304(a) (l) of the Clean Water Act, that are protective of aquatic communities from both acute and chronic effects resulting from exposure to toxic chemicals. EPA is undertaking a comprehensive revision process that will result in the development of two separate methods documents: 1) a Comprehensive Guidelines Document, intended to directly update and expand on approaches presented in the 1985 Guidelines, and will describe methods that provide criteria for chemicals requiring a more detailed level of evaluation, and 2) a Streamlined Guidelines Document, which will focus on criteria development methods that are resource-conserving and can be used to develop scientifically-robust criteria, even when supporting data are more limited. A detailed Scoping Document was recently completed by the EPA, representing a first major milestone in the Guidelines revision process and a basis for subsequent revision activities. The Scoping Document: 1) provides a "roadmap" to the revision process for both the Comprehensive and Streamlined Guidelines Criteria documents, 2) details the specific objectives and approach to the ongoing revision process, and 3) discusses the specific scientific and technical topic areas that EPA has identified for detailed evaluation during the revision and their relevance and applicability to the Comprehensive and Streamlined Guidelines documents. The Scoping Document comprised the focal point of a recent Initial Consultation with EPA's Science Advisory Board, and represents another opportunity for continuing input from the scientific community during the criteria revision process. This presentation provides an overview of the key topics discussed in the completed Scoping Document, preliminary feedback provided by the Science Advisory Board during the Initial Consultation, and ongoing and planned next steps in the Guidelines revision process.

**TP262 Using a Laboratory Food Chain to Assess the Trophic Transfer of Inorganic Selenium into Secondary Consumer *Pimephales promelas***

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Concerns of environmental contamination from selenium have been propelled into the spotlight due to a growing list of disastrous examples of population level declines. The propensity for inorganic selenium species in the aquatic environment to be efficiently bioconcentrated by primary producers and transferred to higher trophic levels primarily via dietary pathways has regulatory agencies grappling for appropriate monitoring strategies. It has been suggested that due to the influence of spatial and temporal variation of the selenium cycle at any ecological site in question, local water quality criteria for selenium should be based on an assessment of site-specific properties. However, the relative importance of biogeochemistry on the uptake and transformation of selenium at the base of the aquatic food chain, and the resulting influence on trophic transfer to higher levels, remains largely unknown. The purpose of this study was to simulate an environmentally relevant aquatic food chain under strict laboratory conditions, to determine the efficiencies of inorganic selenium bioconcentration and trophic transfer from a primary producer to a primary consumer and finally to a relevant secondary consumer species. A green algae species (*Stichococcus bacillarus*) was exposed to graded aqueous selenite concentrations (0, 9, 27 and 54 ug Se/L) and uptake was quantified. The selenium-spiked alga was fed to the primary consumer *Hyalella azteca*, while maintaining the aqueous selenite concentrations. *H. azteca* became the diet for the fathead minnow (*Pimephales promelas*) in a reproductive assay, to determine compartmental distributions of selenium and maternal transfer into the offspring. This study will serve to reduce uncertainty in the trophic processes that relate dissolved selenium to bioaccumulated selenium, crucial for effective regulation for the protection of the environment.

**TP263 Variability of Trace Element Accumulation Among Invertivorous Fishes from a Coastal Plain Stream Contaminated by Coal Combustion Waste**

*D. Fletcher, Savannah River Ecology Laboratory; B.E. Lindell, A.H. Lindell, C.M. Fulghum, J.V. McArthur, University of Georgia / Savannah River Ecology Laboratory*

Invertivorous fishes are an important and diverse component of fish communities in coastal plain stream systems. Species can differ in body form, mouth position, habitat utilization, and feeding strategy. Vertical zone typically inhabited by a species can range from remaining near the water's surface, through being suspended in the water column, to living on or near the bottom. Even among bottom-dwelling species, species may inhabit swift runs, whereas others live in slow pools that represent depositional zones where higher levels of contaminants settle out. Feeding strategy can also influence the amount of sediment ingested. Such factors can influence contaminant exposure and subsequent accumulation resulting in significant variability among fish species. Coal combustion waste contamination exposes aquatic organisms to a broad array of metals and metalloids, consequently patterns of accumulation of multiple elements can be compared among species. We assessed accumulation of 19 elements in over 500 muscle samples distributed across over 25 species of invertivorous fish collected from a coastal plain stream contaminated by coal combustion waste on the Department of Energy's Savannah River Site. Trace element accumulation differed both among and within families. Overall, patterns of trace element accumulation often tended to be species specific.

**TP264 PCB Contamination in Hudson River Surface Water Resources**

*M. Byrne, US Fish and Wildlife Services / Ecological Services; K. Jahn, US Fish and Wildlife Service*

The Hudson River Natural Resource Trustees are determining the natural resource injuries from the General Electric (GE) Company's releases of hazardous substances to the Hudson River from its manufacturing facilities in Hudson Falls and Fort Edward, New York. PCBs released by GE have caused repeated and prolonged exceedances of state and federal water quality standards, contaminating surface water resources of the Hudson River for decades. Since the mid-1970s, federal and state agencies and GE have collected over 10,000 water samples from the Hudson River and have tested these samples for PCBs. Of the 8,667 Hudson River surface water samples that contained PCBs at detectable concentrations, nearly all exhibited PCB concentrations that exceed one or more regulatory standards. Water samples often contained PCBs at concentrations hundreds of times higher than relevant health-protective regulatory criteria for water, such as New York State's 0.00012 ppb regulatory standard to protect wildlife that eat fish, and New York State's 0.000001 ppb regulatory standard to protect human consumers of fish. Approximately 85 percent of samples contained PCBs, often at concentrations an order of magnitude or more above relevant state and federal regulatory criteria. The surface water resources evaluated in this assessment include the Hudson River between Hudson Falls, NY and the Battery in New York City (i.e., all waters below approximately river mile 197). This portion of the river provides habitat for biological resources, including birds, fish, mammals, invertebrates, and plants. The waters and sediments of the river support a diverse ecosystem that includes several species of rare and endangered fish, birds, amphibians, and reptiles. The health of surface water resources is critical to the survival and health of the plants and animals in the ecosystem. In addition, human uses of the river, such as recreational fishing and navigation, are closely linked to the quality of the surface water. The Hudson River Natural Resource Trustees assert these exceedances of water quality standards demonstrate that the Hudson River's surface water has been and continues to be injured.

**TP265 Studies of the physicochemical properties of Ebony River in Ebonyi State, Nigeria and on the abundance of benthic fauna during the raining season**

*A.I. Onwurah, University of Nigeria, Nsukka / Environmental Management; E. Okwuonu, C. Okoye, University of Nigeria, Nsukka / Department of Zoology and Environmental Biology*

The study of physicochemical parameters have been documented, however little may still be known on how those parameters determine the abundance and composition of benthic fauna. We investigated, from May to August, the relationship between physicochemical parameters and the abundance of some benthic fauna found in Ebonyi River. Using a scoop-net, we collected samples ranging from vertebrates and invertebrates. Four sampling stations were chosen so as to encompass as a wide range of physical conditions. The mean values of dissolved oxygen 10.56 (±0.6), alkalinity25.5mg/l (±0.3), hardness 15.3mg/l ($CaCO_3$ ±0.4) were determined. We found out that the abundance of benthos was influenced by physicochemical quality of the water, habitat, and immediate substrate of occupation, tropic condition, resource partitioning and predation. Six classes of both vertebrates and invertebrates were recorded of which the Class Insecta was dominant.Sixteengenera listed in terms of abundance and percentage i.e. *Ephemerella* 0.20(3.16), *Paraleptophilebia* 0.18(2.84), *Macrobrachium* 1.28(20.22), *Sudauonaute* 0.10(1.58), *Coenagrion* nymph 0. 73(11.53), *Aeschnua* nymph 0.20(3.16), *Gyrinus* 0.38(6.00), *Sympetrum* 0.90(14.22), *Lestes* 0.20(3.16), *Dytiscus* 0.05(0.79), *Hlobdella* 0.15(2.37), *Belostoma* 0.08(1.26), *Viviparus* 0.45(7.11), *Tilapia* 0.23(3.63), *Gomphoide* 0.50(7.90), *Chironomus* 0.70(11.06) were observed. The genera of *Coenagrion* nymph, *Sympetrum*, and *Chironomus* negatively correlated to the depth and velocity of water. However, we found a positive relationship between the water temperature and *Belostoma* genus. The negative relationships may be as a result of pollutants acquired through anthropogenic activities. However, the positive relationship between temperature and *Belostoma* genus may be as a result of essential resources e.g. food and mating season.

## Fate, Toxicology, or Risk Assessment of Materials of Interest to the Military

### WP001 Predicting Transformation Products and Physicochemical Properties of Insensitive Munition Constituents

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Traditionally, the defense industry has focused on the use of explosives such as TNT, RDX, and HMX but handling and use of these chemicals is associated with high risk of unintended detonation. Recently there has been interest in many countries, especially the United States, in using new insensitive munitions constituents (IMCs) to replace traditional explosives in the manufacturing of munitions formulations. The development and increased utilization of IMCs is creating a growing demand for information on their potential environmental impacts. To provide a complete risk assessment of IMCs, it is necessary to consider their *transformation products*. This project aims at providing a more comprehensive basis for assessing and managing the environmental risks of IMCs by extending current fate and transport models for soils and groundwater to include the products of IMCs transformation. For this, the approach includes: (*i*) determination of IMCs transformation pathways and products, (*ii*) determination of the fate determining properties of IMCs transformation products, and (*iii*) development of models that describe the fate and transport of IMCs transformation products. Presently, we are incorporating prediction library rules from existing models for expected transformation mechanisms (e.g., hydrolysis and nitro-reduction) into a novel machine-learning-based software for the estimation of IMCs transformation products along with the corresponding reaction thermodynamics. Initial results with two of the main IMCs (2,4-DNAN and NTO) show that we are able to predict the reduction products. We are also using molecular structure to estimate IMCs fate determining properties including water solubility and octanol-water partition coefficients. Preliminary estimation errors are as low as 0.5 log units from the experimentally measured values. The results of this project have practical and general applications. The practical benefit of this work will be to enable DoD training site managers to include consideration of (I)MCs transformation and products into the modeling they do for evaluation of exposure assessment scenarios, risk, remediation, etc. General applications of the results from this project will be through advancement of methods for prediction of transformation pathways, chemical properties, and reactive- transport models that could be adapted for assessing the environmental fate of other contaminant classes also of interest to the military (e.g., PFASs).

### WP002 Toxicity of the Insensitive Munition 2,4-dinitroanisole to the Earthworm *Eisenia andrei*

*M. Simini, US Army Edgewood CB Center / Environmental Toxicology; R.G. Kuperman, Edgewood Chemical Biological Center / Molecular Toxicology Branch; G.R. Lotufo, US Army ERDC / Environmental Laboratory*

The Department of Defense is developing and evaluating several insensitive munitions (munitions that will not react violently in an accident situation) for future weapon systems to replace present munitions that contain highly sensitive explosives, such as 1,3,5-trinitro-1,3,5-triazine (RDX) and 2,4,6-trinitrotoluene (TNT). Among these compounds is the insensitive munition (IM) compound 2,4-dinitroanisole (DNAN), for use as a component in several IM explosive compositions (IMXs). Data are needed to develop ecotoxicological thresholds for IM compounds that can be used to derive risk-based regulatory levels for selected key soil ecological receptors. The present study used the earthworm toxicity test with *Eisenia andrei* (ISO 16387:2004) to determine DNAN toxicity in a natural soil that has a "very high" qualitative relative bioavailability score for organic chemicals. Adult earthworms (0.3 to 0.6 g each, five per container) with fully developed clitella were randomly selected and placed in glass containers filled with 200 g of test soil. Two grams of worm food were placed in each container. A piece of clear plastic film was stretched over the top of each container and secured in place with a screw top. Pinholes were made in the plastic film to facilitate air exchange. Four replicates were prepared for each treatment level and controls. Adults were removed and counted after 28 d. Juveniles and cocoons were removed and counted after 56 d from the start of exposure. The range-finding study yielded EC20 and EC50 values of 277 mg/kg and 351 mg/kg DNAN for adult survival, respectively, and 72 and 98 mg/kg DNAN for cocoon production, respectively. Results of the range-finding assay was then used to establish the range of values used for the definitive assay. Results of the definitive assay will be reported and the toxicological endpoints will be used together with results from other invertebrate toxicity assays to develop Ecological Soil Screening Levels (Eco-SSL) for IMs that meet regulatory requirements for screening level ecological risk assessments (SLERA), plus will meet technical requirements for developing risk-based soil threshold levels for regulatory compliance in baseline ecological risk assessment (BERA).

### WP003 Ecotoxicological Effects of Insensitive Munition Compound 2,4-dinitroanisole in Soil on Potworm *Enchytraeus crypticus*

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We are investigating the ecotoxicity of an insensitive munition compound (IM) 2,4-dinitroanisole (DNAN), developed for future weapons systems to replace present munitions that contain highly sensitive explosives. We designed our studies to meet regulatory requirements for developing Ecological Soil Screening Levels (Eco-SSL) for use in screening-level ecological risk assessment by adapting the standardized toxicity test for the soil invertebrate potworm *Enchytraeus crypticus* (ISO/16387:2005) using exposures in Sassafras sandy loam (SSL). This soil has a "very high" qualitative relative bioavailability score for organic chemicals in natural soils. Studies include potworm exposures to DNAN freshly amended into soil (after 24-h moisture equilibration period), or to DNAN subjected to wetting-and-drying cycles in soil in a greenhouse. This is done in order to determine toxicity benchmark values for DNAN in soil for each exposure type; including simulation of DNAN weathering-and-aging fate processes that occur in the field. Survival and reproduction data were analyzed in the preliminary study using nonlinear regression models to determine DNAN concentrations producing a specified percent effect (e.g., 50%) on the measurement endpoints. Both adult survival, and juvenile production were affected by *E. crypticus* exposure to DNAN in SSL soil based on the LC50 and EC50 nominal concentrations (mg/kg) 143 and 68, respectively, in the freshly amended treatment. These results were used to selected concentrations for the ongoing definitive toxicity tests. When completed, these studies will fill the existing data gap in current knowledge regarding the potential ecological risks of release of DNAN into soil.

### WP004 Bioaccumulation of the Insensitive Munition Compound 2,4-dinitroanisole from Soil on the Earthworm Eisenia andrei

*G.R. Lotufo, R.E. Boyd, US Army Engineer Research and Development Center / Environmental Laboratory; A. Harmon, US Army Engineer Research and Development Center; R.G. Kuperman, Edgewood Chemical Biological Center / Molecular Toxicology Branch*

We are investigating the bioaccumulation potential of an insensitive munition compound (IM) 2,4-dinitroanisole (DNAN), developed for future weapons systems to replace present munitions that contain highly sensitive explosives. We exposed the earthworm *Eisenia andrei* to Sassafras sandy loam (SSL) soil freshly spiked with sublethal concentrations of DNAN. This soil has a "very high" qualitative relative bioavailability score for organic chemicals in natural soils. Formation of 2- and 4-amino-nitroanisole (2A-4NAN and 4A-2NAN) occurred in spiked soils, but never at concentrations higher than that of DNAN. SumDNAN (sum of DNAN, 2A-4NAN, and 4A-2NAN) in soil decreased dramatically (up to 80%) during the exposure period (up to 21 d) in the presence of earthworms or plants, likely driven primarily by irreversible binding of aminated transformation products. Overall, SumDNAN body residue of earthworms remained relatively constant from 7 to 14 d, indicating relatively short time to achieve steady state. Because of the reported short half-life of DNAN in earthworms tissues, bioaccumulation factors (BAFs: ratio of the tissue to soil concentration) were determined in worms non-purged for gut contents, but corrected for the dilution effect of soil in the gut. Mean 14-d BAF were 17.6, 4.9 and 4.4 for DNAN, 2A-4NAN and 4A-2NAN, respectively. These results were used to select exposure parameters for ongoing definitive bioaccumulation tests

**WP005 Review and Synthesis of Evidence Regarding Environmental Fate and Risks Posed by Energetic Components at Underwater Munitions Sites**

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We investigated the degree to which underwater military munitions (UWMM) pose a risk to the aquatic environment. UWMM may contain energetic compounds (EC) such as 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), which are among the most widely used high explosives. If UWMM becomes corroded or breaches, the fill material may leak or dissolve into the surrounding environment, potentially adversely affecting the exposed biota. In large part because of the high cost and complexity associated with sampling EC at UWMM sites, detailed and reliable information about MC in water, sediment and biota is available for only few sites and temporal and spatial uncertainties persist. Our compilation and examination of available data revealed that EC concentrations in water and sediment were largely below detection, with a few samples indicating contamination as highly localized and typically near the UWMM. To complement available site data, fate studies predicted very low concentrations of EC in the water column at UWMM sites. Available toxicity data derived for freshwater and marine fish, invertebrates and autotrophs was compiled and species sensitivity distributions were derived. Risk to biota was determined to be low at UWMM sites when measured or modeled site concentrations were compared to toxicity data.

## Using Non-Conventional Animal Models in Ecotoxicological and Genotoxicological Studies for Risk Assessment Applications

**WP006 Development of chronic water-only toxicity testing methods using mayflies**

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*Hexagenia* is a genus of burrowing mayfly that is increasingly used in sediment toxicity and bioaccumulation assays. Although this genus is primarily used in sediment bioassays, there is a desire by regulatory agencies to conduct water-only experiments with *Hexagenia*, as it reduces the complexity and additional considerations associated with a sediment test. Furthermore, the ability to compare *Hexagenia* toxicity data to that of other aquatic invertebrates would improve their utilization in water quality assessments. While acute (96 h) water-only tests are routinely conducted, there has been limited success with chronic (21 d) exposures. Therefore, to fully utilize *Hexagenia* in water quality assessments, a chronic method needs to be established. The objective of this study was to establish a method to maintain these organisms for 21 d with acceptable (< 20%) mortality. Several types of artificial burrows, as well as several substrates, feeding regimens, and light conditions were evaluated. It was determined that adding clean sea sand in conjunction with the standard glass artificial burrows yielded survival above acceptable levels. Furthermore, survival was acceptable in light and dark exposures and with two different feeding regimens. Additionally, survival rates were compared among methods in the presence of a chemical stressor (KCl) to determine if one method yields higher survival. The selected method will be validated by multiple laboratories that are interested in chronic water-only mayfly exposures, so ultimately these methods can be implemented by these agencies.

**WP007 Invertebrate Cardiotoxicity: Investigating the effect of Phenanthrene on the heart function of signal crayfish *Pacifastacus leniusculus***

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Crustaceans are regarded as excellent bio-indicators in the aquatic environment but little is known about their response to pollutants such as polyaromatic hydrocarbons (PAHs). Most recent studies focus on the impact of PAHs on marine fish and zebrafish where they have implicated phenanthrene (Phe) as the cardiotoxic PAH. This study aims to investigate the effect of Phe on the cardiac function of the invasive signal crayfish. Using a semi-static renewal method, 40 signal crayfish were exposed to 0, 0.5, 1.0, and 1.5 mg/L of Phe for fifteen days at 10°C. Heart rates (HR) were monitored every 48 hours using a non-invasive infrared sensor glued to the carapace. The crayfish were first stressed to elicit maximum HR and then allowed to recover. Prior to exposure, a baseline standard recovery curve for HR was obtained. After the exposure period, the monophasic action potential (MAP) and electrocardiogram (ECG) were recorded in a semi-isolated heart which was constantly perfused with aerated crayfish saline at 10°C. In vivo HR recovered ($P< 0.001$) to baseline in 60 mins after stress in all groups. Time-dependent analysis of HR recovery indicted that during early exposure (1-7 days) recovery of HR was significantly prolonged by up to 30 mins in the 0.5mg/L group and by 45 mins in the 1.0mg/L group resulting in a significant delay recovery time to 75%. In all cases, elevated HR returned to baseline after 60 mins. This early exposure effect in the 1.0mg/L group corresponded to a significant reduction in the detected peak-peak (N-N) interval from the smoothened HR signal. Late exposure (8-15 days) caused a significant reduction in max HR in the mid and highest concentrations thereby causing a slight downward shift of the recovery curve from the baseline. Heart rate variability (HRV) significantly increased in the 0.5mg/L and 1.5mg/L group within the last 15mins of recovery in the late exposure phase. Chronic Phe exposure (15 days) was found to affect the electrical properties of the heart by causing prolongation of action potential duration (APD90) and significantly ($P< 0.01$) elongating the corrected QT (QTc) interval. This study is consistent with findings from marine vertebrates upon exposure to PAHs. Understanding the cardiac response of crustacean heart to PAHs and physical stressors could be vital in cardiophysiological studies.

## What Matters: Global Perspectives in Advancing Science into Environmental Risk Assessment

**WP008 Ecological Risk Assessment of Microplastics and Associated Contaminants in American Samoa**

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Solid waste disposal and recycling is a massive concern among Pacific Island nations. For this reason, the Marine Debris Program of the American Samoa Environmental Protection Agency has prioritized plastics monitoring, research and risk assessment within their 2016-2018 strategic objectives. We applied an ecological risk assessment framework to: 1) quantify the type and concentration of microplastics in water, sediment and biota on the island of Tutuila, American Samoa; 2) determine the type and concentration of hydrophobic organic contaminants in microplastics, waters, sediments and biota at several study sites; and 3) estimate toxicological impacts of microplastic and organic contaminant pollution on locally consumed bivalves and fishes, in order to characterize ecological risk to marine ecosystems and health risk to human populations. Although many organic contaminants and large areas of plastic debris were detected and/or recorded, microplastics were less frequently encountered in the environment and biota. Results will inform ongoing environmental regulation, educational outreach, and marine conservation efforts in American Samoa. As seafood is an important source of protein for other populations around the world, this study also provides a framework for community, scientific or regulatory agencies working in data-poor regions to conduct screening-level risk assessments using in-situ, baseline studies at the local or regional scale.

**WP009 Clear, Complete, and Justified Problem Formulations for Aquatic Life Benchmark Values: Specifying the Dimensions**

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Nations that develop water quality benchmark values have relied primarily on standard data and methods. However, experience with chemicals such as Se, ammonia, and tributyltin has shown that standard methods do not adequately address some taxa, modes of exposure and effects. Development of benchmark values that are protective requires an explicit description of the issues, a problem formulation. In particular, the assessment endpoints and other dimensions should be specified for each chemical so that the necessary data will be obtained and appropriate analyses will be performed. Assessment endpoints specify the entity and attribute to be protected. In addition, the level of protection, including the magnitude of effect and the proportion affected is specified. Magnitude and proportion are included, because they are used to calculate the benchmark concentration. If uncertainty is considered in the benchmark, the proportion of the uncertainty distribution that is protected should be specified. Because effects are related to the duration of exposure and time for recovery, temporal dimensions should be specified. Clearly described exposure metrics are also needed, because the relevant exposure parameter is not always total aqueous concentration. Finally, the benchmark may be applicable to particular geographic or climatological areas, water chemistries, taxa, or habitat types. Considering and justifying all the dimensions is likely to result in protective and more easily communicated benchmarks. The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the USEPA.

**WP010 Evaluating Regional Variability in Consumer Product Consumption Data for Environmental Exposure Assessments**

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Consumer product manufacturers conduct prospective risk assessments to ensure safety of their products and to meet regulatory requirements across the globe. Exposure is based on the use of the product as designed, but priorities may differ between geographical areas. From an environmental perspective, exposure is often based on assumed down-the-drain emission. Understanding the quantity of chemical emitted is a necessary component of calculating exposure and the calculation options will be evaluated for alignment. Either annual tonnage or daily consumer consumption data can be utilized as inputs for this calculation. Annual tonnage may be assessed through internal shipping data, estimated marketing information, or external market data. Consumption rates may be estimated through consumer habit and practice surveys, or by utilizing market data from third party agencies. Additionally, opportunities and limitations of the applicability of the data sources, and implications for use will be assessed. By evaluating global sources of tonnage and consumer consumption data regional differences in product use will be identified to enhance the relevancy of the estimated emission values for use in environmental risk assessments.

**WP011 Practical Considerations for the Incorporation of Insect-Mediated Contaminant Flux into Ecological Risk Assessments**

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The approach to the management of environmental contamination has progressed as our understanding of the factors that influence the fate and effects of contaminants has evolved. Presently, most contaminated sites are managed under the widely applied risk assessment framework, which was designed not as a rigid structure but rather as a flexible process that could be modified over time and used in multiple contexts. Our scientific understanding has now advanced to the point that risk assessors can apply ecological interactions at the land-water interface, specifically insect-mediated contaminant flux, within the current framework of ecological risk assessment. In this talk, a conceptual model for insect-mediated contaminant flux will be described and its implication for the fate and transport of environmental contaminants, as they move from the aquatic ecosystem to the terrestrial environment, will be discussed. Additionally, a novel practical stepwise pathway, The Riparian Impact Test, will be introduced to help risk assessors determine if adult aquatic insects are a potential concern for the re-distribution of aquatic contaminants to the terrestrial ecosystem at specific contaminated sites.

## Environmental Pollution in Developing Economies, Policies and Population Dynamics

**WP012 Assessment of Toxicological Impact of Anthropogenic activities on Onitsha Stretch of River Niger in Southeastern Nigeria**

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River Niger is one of the eight major rivers in Nigeria. This river flows through a heavily populated and commercialized city of Onitsha in Southeastern Nigeria. It also aids various agricultural, commercial, domestic activities. Waste from different anthropogenic sources and channelled into the river. However, information on toxicological and water quality status of Onitsha stretch of River Niger is scarce. Therefore, the aim of this study was to assess the toxicological impact of mixtures of chemical substances on water quality of River Niger. Water samples were collected from three sampling stations. Station 1: Niger-bridge sampling point (NBSP), Station 2: Nwangele Lake sampling point (NLSP) and Station 3: Marine Police sample points (MPSP). With a sterilized pre-labelled 2 litres' sample container. The water sample were analysed for physical and chemical parameters, heavy metal content and endocrine disrupting compounds (EDCs) in water. Heavy metal pollution index (HPI) was calculated for each parameter (Reza and Singh, 2010; Prasad and Mondal, 2008; Prasad and Kumari, 2008). Metal Pollution index (MI) was also calculated. MI value I is a threshold of warning (Mohan, et al 1996; Prasad and Kumari, 2008). Six heavy metals were obtained from Onitsha stretch of River Niger, the heavy metal pollution index obtained in the different sampling points were far above the critical values of 100 and the metal index value obtained were also higher when compared to the values reported by Lyulko et al 2001. The concentration of heavy metal obtained in the different sampling points based on the range and abundance are ranked Zn>Pb>Hg>Cd>Cr>Ni. The values obtained for heavy metal pollution index from different stations NBSP (5425), NLSP (6268) and MPSP (6420) and the metal index from the stations NBSP (428.55), NLSP (531.32) and MPSP (651.7) were far above the critical values 100 used in assessing pollution (Lyulko et al 2001). The endocrine disrupting compounds found in the study area include: polycyclic aromatic hydrocarbon, phthalates, polychlorinated dibenzo-*P*-Dioxins (PCDDs), Polychlorinated dibenzo furans (PCDFs), polybrominated diphenyl ethers (PBDEs), bisphenol A and polychlorinated biphenyl (PCBs) were obtained at varying concentrations.

**WP013 Blood Lead (Pb) Levels and Stable Isotope Ratio among Children, Infants and Mothers in Kabwe, Zambia**

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Lead (Pb) poisoning is a serious human health concern, and one of the most common and best-recognized childhood diseases of toxic environmental origin. Lead poisoning is entirely preventable, yet it accounts for about 0.6% of the global burden of disease, with the highest burden in developing countries. Kabwe town, capital of the Zambian Central Province with a population of approximately 203,000, is among one of the most polluted places in the world. The town has a long history of lead-zinc mining, which operated for nearly 100 years without any pollution laws regulating emissions from the mine, and addressing the potential dangers of Pb contamination. Despite closure of the mine, scavenging of metal scraps from the abandoned tailings, use of lead-laced soil to make bricks, dust emanating from the mine dumps, etc… have continued to serve as a source of metal pollution. This present study investigated blood lead levels (BLLs) and stable isotope ratio in children and infant-mother pairs living around and far from the closed Pb-Zn mine in Kabwe. In total, 153 children's and 417 infants'-mothers' paired blood samples and fecal samples from infants were collected. BLLs were measured using the Lead Care II analyzer System. The result showed the prevalence of Pb poisoning in the townships near to the mining area. 93% children and 77% infants BLLs exceeded the CDC recommended level of 5mg/dL. Furthermore, ten blood samples from infants exceeded the level that can cause encephalopathy and even death (100 µg/dL). A significant correlation between BLLs of paired mothers and infants was detected. This clearly demonstrates the ability of Pb to transfer via breastfeeding. Stable isotope ratio measured with Multi-collector ICP-MS suggested that infants with high BLLs were exposed via soil and/or dust. In summary, mothers and infants with high BLLs, children who play in the soil, and young men and women who artisanally mine in the area are most susceptible to Pb and, therefore, they are at high risk. Finally, it is recommended that periodic monitoring of blood Pb in infants and children, and educating the local population should be undertaken to control the Pb blood levels in the whole population.

**WP014 Describing local air quality problems using an ontological framework: The case of Chile**

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Air quality problems arise from a combination of the emission of contaminants and meteorological factors that affect their transport and dispersion in the atmosphere. According to the International Agency for Research on Cancer (IARC), air quality could be responsible for the increase in mortality/morbidity rates for many chronic human diseases such as cancer. Local governments and international agencies have therefore declared air quality as a major concern for human health. Air quality regulations in Chile started in 1998 and have historically been based on the daily-average concentration of particulate matter (PM) 2.5 and 10 mm. Local environmental alerts and emergencies are declared when $PM_{2.5}$ or $PM_{10}$ measured in the state-run monitoring network are above 50 or 150 mg/m$^3$ respectively. Despite these efforts, several Chilean cities including the Santiago Metropolitan Region still have air quality problems. Additionally, other southern cities in the country are now among the most polluted in the world, due to the extensive use of wood for heating purposes; while cities in the north show recent increases in health risks associated to atmospheric pollutants although having lower-than-average PM concentrations. These facts show us that there are other important areas in the air quality management strategy that need to be developed. This study aims to explore these missing areas in Chile. The first part focused on the description of our main topic "Air Quality in Chile". This was based on the opinion of a panel of experts with different academic backgrounds (environmental scientists, public health, sociologists). The results were organized in an ontological framework to describe the topic in a systematic, systemic and logic way, by dividing it into several categories and dimensions. The objective here was to comprise all the important aspects related to the Air Quality problem in Chile in an easy-to-visualize framework. The second part of this study focused on the meta-analysis of the existing literature derived from our main topic. We classified all the articles found using our ontological framework previously developed and identified several missing areas in the current state of the research. This will help researchers and government funding institutions to prioritize their efforts to cover these missing areas. The results from this study will also be used in the analysis of the current state of the public policy in the country.

**WP015 Effect of organic and inorganic fertilizers on the bioremediation of used motor oil polluted soil**

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Three treatments (poultry manure (PM), Nitrogen Phosphorus Potassium fertilizer (NPK), and a combination of both) were used for bioremediation of soil spiked with used motor oil to determine the potential of these treatments in enhancing biodegradation of used motor oil in soil. The degree of biodegradation of the oil was studied for a period of 4 weeks under laboratory conditions. Hydrocarbon-utilizing bacteria counts were high in all the poultry manure-amended soil ranging between $9.0\times10^6$ and $30\times10^6$CFU/g compared to unamended control soil throughout the 4 weeks of study. Oil contaminated soil amended with a combination of poultry manure and NPK fertilizer showed the highest reduction in total petroleum hydrocarbon with loss of 80% in the 4th week compared to other treatments. The results obtained demonstrated the potential of the treatments for oil bioremediation in the order: Poultry Manure + NPK > Poultry Manure > NPK.

**WP016 Effect of textile mill effluent on the growth of Zea mays**

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The indiscriminate disposal of effluents from textile and chemical industries in Nigeria has become a serious problem to the environment. Most of these effluents are disposed into farmlands or directly used for irrigation purposes. This research investigated the effects of textile mill effluent on the germination and growth of Zea mays. Effluent samples were collected from a textile industry and the physicochemical parameters were analysed. Five viable maize seeds each were planted in wood shavings (sawdust) irrigated with different concentrations of textile effluent (0, 25, 50, 75 and 100 %) and observed for 15 days. Results of the physicochemical analysis showed that most of the parameters were above permissible standards. Parameters like BOD (171), COD (4208) and Nitrate (71.2) were found to be very high above the permissible limits. There was complete loss of viability at concentration 100%, while germination reduced by 75, 50 and 25% in 75, 50 and 25% textile effluent concentrations respectively. Plants growth rate was inversely proportional to concentration increase, the growth of the control significantly differed from other treatments at $p < 0.05$.

**WP017 Environmental heavy metals exposure: Impact on blood levels of heavy metals in development of cardiac diseases in ESUTH, Enugu, Nigeria**

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Substantial heavy metals exposure lingers in emerging economies such as Nigeria, where existing environmental and industrial policies/regulations are not firmly enforced. Despite indications that environmental heavy metals (Lead, Arsenic, Cadmium and Mercury) exposure may play important role in the pathogenesis of Cardiac Diseases, it has not been investigated in human population from a developing economy like Nigeria. The aim of this study is to determine the role of blood Lead, Arsenic, Cadmium and Mercury in development of cardiac diseases. Blood Mercury, Arsenic, lead and cadmium levels were determined by Atomic Absorption Spectrophotometry (AAS) in 20 male Cardiac disease patients, 20 female Cardiac disease patients, 20 male control subjects and 20 female control subjects. There was significant increases in Blood Arsenic (As), lead (Pb) and cadmium (Cd) ($p=0.000$, $p=0.000$ and $p=0.005$ respectively), but no significant difference in BMI and blood mercury ($p=0.289$ and $p=0.593$ respectively) in all Cardiac disease patients compared to controls. There was significant increases in Blood Arsenic (As), lead (Pb) and cadmium (Cd) ($p=0.001$, $p=0.002$ and $p=0.040$ respectively), but no significant difference in BMI and blood mercury ($p=0.239$ and $p=0.031$ respectively) in male Cardiac disease patients compared to male controls. There was significant increases in BMI, blood Arsenic (As), lead (Pb) and cadmium (Cd) ($p=0.02$, $p=0.008$, $p=0.017$ and $p=0.010$ respectively) in female Cardiac disease patients compared to controls. There was no significant difference in blood Mercury (Hg), Arsenic (As), lead (Pb) and Cadmium (Cd) ($p=0.208$, $p=0.517$, $p=0.155$, and $p=0.243$ respectively) in male Cardiac disease patients compared to female Cardiac disease patients. There was positive correlation of blood lead (Pb) with Cadmium (Cd) and Mercury (Hg) ($r=0.474$ $P=0.036$ and $r=0.492$ $P=0.028$) in all Cardiac disease patients, but there was no significant correlation of blood lead (Pb) with BMI and blood arsenic (As) ($r= -0.104$ $P=0.490$ and $r=0.088$ $P=0.712$ respectively). It therefore appears that increases in blood levels of Arsenic, lead and cadmium due to environmental exposure may in part contribute to the development of Cardiac diseases in this environment. Thus strict measures should be applied by the policy makers to reduce heavy metals pollution in Nigeria.

**WP018 Metal accumulation and human health risk associated with the consumption of *Oreochromis mossambicus* and *Labeo rosae* from Loskop Dam**

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Loskop Dam is known as a repository for contaminants from the entire upper Olifants catchment. Its fish populations have shown elevated metal concentrations within tissues. These concentrations have become a cause for concern as local communities rely on fish from Loskop Dam for protein supplements. This study was carried out to measure metal concentrations in *Oreochromis mossambicus* and *Labeo rosae*, and to assess edibility. Water and sediment samples were collected during winter and summer 2014 at Loskop Dam. Concurrently with water and sediment sampling, ten specimens for each specieswere collected. Metals were found to be below detection levels in the water column, however, significantly high concentrations were observed in sediment. *Labeo rosae* has shown hazard quotient of >1 for arsenic and lead, with selenium and antimony showing a hazard quotient of >0.5. For *O. mossambicus*, arsenic showed a hazard quotient of >1, with selenium and antimony showing a hazard quotient of >0.5. Lead concentration was significantly low in the muscle of *O. mossambicus*. Sediment has shown significantly higher concentration of metals as compared to fish tissues. Given that metals fixed in sediment may remobilise back into the water column, it is thus, predicted that metal concentrations in fish tissues will increase in the near future. The findings of this study serve as an alerting measure and highlight the need for recommendations and urgent interventions to reduce metal enrichment at Loskop Dam due to the serious human health implications.

**WP019 Policy management gaps, bromate content in bread and human health**

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Compliance to the existing laws in Nigeria with respect to food production by manufactures is grossly inadequate due to poor monitoring and supervision by regulatory bodies saddled with these responsibilities. Despite the fact that bakers are ban from using bromate in baking bread, there is a lot of suspicion that this compound is still being used by many bread industries. Therefore, increased risk due to consumption of bread with some levels of bromate, a possible carcinogen, by the populace may be inevitable. Hence this study estimated the levels of bromate in some bread samples from Enugu State Nigeria. A total of 30 brand of bread samples were purchased at major residential areas in Enugu Nigeria using simple random sampling by balloting without replacement three times from the same locations at intervals of two weeks. The results showed that bromate ranged from 14.93-24.28mg/kg in all the bread samples collected. These levels of bromate in the bread samples highly exceeded the United States Food and Drug Agency of 0.02 mg/kg (0.02µg/g) acceptable level in human system. Therefore, individuals who consume bread marketed in this region are at high risks of cancer, kidney damage, aural dysfunction, bronchial problems amongst others due to bio-accumulation. Furthermore, the presence of bromate in the bread samples indicates that the substance is still being utilized by bread producers despite the ban on its use by NAFDAC. The need for routine checks and sanctions of culprits is highly recommended and there is need for food safety awareness for the general public/ in the food market.

**WP020 Public health concerns of bromate in bread samples in Nigeria**

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INTRODUCTION:Universally, bread is commonly consumed as a snack or an accompaniment to main meals. Bromate is normally utilized to make breads generally appealing to consumers. However, the International Agency for Research on Cancer (IARC) has labeled potassium bromate ($KBrO_3$) as a carcinogen. This study evaluated the total bromate in bread samples consumed in Nsukka Nigeria. METHODS 10 bread samples were purchased at regular intervals of two weeks for three times at the same locations until a total of 30 bread samples were purchased. Standard methods were used to determine the total bromate in the samples. Statistical software (SPSS version 21) was used to calculate the means and standard deviations. FINDINGS AND INTERPRETATIONS The results indicated that all the bread samples had bromate in concentrations above safe levels for human consumption (0.02mg/kg limit by US Food and Drug Agency). The bromate level of the samples ranged from 39.52-154.95mg/kg and the average concentration was 58.8mg/

kg. Such high levels of bromate are of public health concern as individuals are vulnerable to debilitating health conditions reported for bromate consumption. More so, the potential of bromate to degrade some vitamins in bread militates/opposes the global goal of combating micronutrient malnutrition. Nutrition communication and education of the general public is essential for unsuspecting vulnerable bread consumers to be aware of the safety of the foods they eat. Closure of bakeries utilizing bromate and other strict measures should also be employed. CONCLUSIONS: This research had shown that most bromate freelabelled breads in the market are not totally free and safe for human consumption. Hence, the bioaccumulation may lead to non- communicable diseases such as cancer. Key words: Public health, bromate,nutrition, bread.

**WP021 Regulatory Policies and Environmental Compromise in Developing Nation: The Nigerian Experience**

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The role of various regulatory bodies in the control of food, drugs and other consumables is a vital component of safeguarding the health of the populace. However, a review of these activities shows that there is a strong negative impact on the environment. This work therefore evaluates the activities of some regulatory bodies in Nigeria between the years 2000-2017. Some of the activities examined include policy implementation and methods. Three major bodies were examined; Standard organization of Nigeria (SON), National agency for food administration and drug control (NAFDAC) and Nigerian Drug and law enforcement agency (NDLEA). Over 4000 reported cases were randomly selected from online sources and print media. The result shows that open incineration (75%) and shallow landfills (25%) are the common methods used in disposing seized commodities. These activities increased from year 2000 to 2017 but in 2017 we had the most significant amount of destroyed products both in volume and in cost of about 1.3 trillion naira. However, a total of 43 billion naira goods were either burnt or buried in the soil. Similarly, it was also observed that seized items were not sorted out neither were any laboratory analysis carried out on these items before incinerating them to ascertain the specific elements present for guided disposal action. Although the rate of boarder smuggling is on the increase, continuous open burning and burying of seized items may lead to future public health issues. We therefore conclude that regulatory bodies in developing nation should balance policies, activities and environmental safety.

**WP022 Risk assessment and estimation of total bromate in bread marketed in Enugu State, Nigeria**

*L.U. Iyeghe, University of Nigeria, Nsukka / Home Science, Nutrition and Dietetics; O. Otitoju, Federal University, Wukari / Biochemistry Department*

Compliance to the existing laws in Nigeria with respect to food production by manufactures is grossly inadequate due to poor monitoring and supervision by regulatory bodies saddled with these responsibilities. Despite the fact that bakers are ban from using bromate in baking bread, there is a lot of suspicion that this compound is still being used by many bread industries. Therefore, increased risk due to consumption of bread with some levels of bromate, a possible carcinogen, by the populace may be inevitable. Hence this study estimated the levels of bromate in some bread samples from Enugu State Nigeria. A total of 30 bread samples were purchased at major residential areas in Enugu Nigeria. Simple random sampling by balloting without replacement was used to select 10 bread samples and each sample was purchased three times from the same locations at intervals of two weeks. The results showed that bromate ranged from 14.93-24.28mg/kg in all the bread samples collected. These levels of bromate in the bread samples highly exceeded the United States Food and Drug Agency of 0.02 mg/kg (0.02µg/g) acceptable level in human system. Therefore, individuals who consume bread marketed in this region are at high risks of cancer, kidney damage, aural dysfunction, bronchial problems amongst others due to bio-accumulation. Furthermore, the presence of bromate in the bread samples indicates that the substance is still being utilized by bread producers despite the ban on its use by NAFDAC. The need for routine checks and sanctions of culprits is highly recommended and there is need for food safety awareness again for the general public in the food market.

**WP023 Risk Associated with Alternative Cleaning Method for Carrots**

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A carrot is a nutritional root vegetable which is loaded with beta carotene, a precursor of vitamin A (Kotechal *et al*., 1998; Speizer *et al*., 1999). it is necessary to wash carrots in order to remove soil and other foreign materials before eating either raw or in processed form (Moos *et al*., 2002). It is common practice today to soak carrot in detergent solution before washing to achieve better cleaning. Studies have shown that some of the components of detergent are toxic (Jerome *et al*., 2003; HERA, 2013; Rima *et al*., 2006; Chuku *et al*., 2015). This work is therefore aimed at evaluating the residue accumulated in carrot washed with detergent. The study was divided into two stages. In the first stage, questionnaires were distributed to determine the popularity of the use of this chemical substance in washing carrot before selling to consumers. The second stage involved weighing out 2 kg of fresh carrots and soaking them in thirty one different containers. Five containers were used for increasing concentration (0.5, 1.0, 1.5, 2.0 and 2.5 g/L) of detergent 1 (DET 1) while another five containers were used for same concentrations of DET 2. The set up was allowed to soak for 20, 40 and 60 minutes. The control group was soaked in distilled water. At the end of the soaking period, the carrots were dried, grounded into powder and then analyzed using the titrimetric method described by IPAN (2005). Results obtained showed that 64.29% of the respondents agreed to the use of detergent in soaking carrot before washing, 25.14% do not use detergent in washing their carrots before selling to consumers while 10.57% were indifferent. The study also showed that the percentage anionic surfactant in carrot soaked in detergent at different time intervals before washing increased significantly ($P < 0.001$) as the concentration of detergent added and the exposure time increased. There was also a slight increase in the amount of cationic surfactant residue deposited in the carrot. On the whole, DET2 concentrated more of the cationic detergent residue than DET1. The presence of residual amount of detergent in the exposed carrot raises a public health concern as this food item is consumed daily by unsuspecting public.

**WP024 Using linear alkylbenzenes (LABs) to identify anthropogenic contributions to aquatic pollution in complex ecosystem**

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Identifying contaminants inputted to the environment and tracing anthropogenic contributions from various socio-economic activities are desirable for implementing effective management and remediation strategies. Linear alkylbenzenes (LABs) have been used as molecular markers to identify the potential anthropogenic origins for other organic pollutants with similar physiochemical properties, such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). Linear alkylbenzenes are usually discharged in the environment as residues of commercial detergents and closely related to socio-economic activities during urban development. The different sources of LABs were investigated in this framework, with the use of factor decomposition method and experimental data. The cities located in the Pearl River Delta (PRD) with one of the highest population densities and fastest economic growth rates in China are mainly responsible for production and discharge of aggregated pollutants in the aquatic system. The framework identified the direct (including urban and rural domestic sewage and industrial wastewater) and indirect contaminant sources (including the external input and output and agricultural irrigation). The results showed that LABs inputted to the environment from socio-economic activities accounted for 2363.8 t/yr of Guangdong province and about 1325.3 t/yr of PRD in 2014. Domestic sector contributed 56% of the total LABs discharge, Industry and public service sectors both accounted for 41% of the total. From 2004 to 2014, LABs increased 1968 t and LABs concentration in industry sewage and consumption per capita were the main contributors to the increase of LABs. The sources of other organic pollutants will be discussed by applying the framework and comparing with the percentage of LABs. Identification of environment pollution liability is an important part of integrated pollution control in complex ecosystem. The current research provides theoretical support and a database for the formulation of policies leading to regional organic pollutant reduction and remediation of pollution.

**WP025 Effects of anthropogenic activities on physicochemical characteristics and benthic macroinvertebrates in streams in Dschang, West Region of Cameroon**

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In Africa many water bodies are subjected to anthropogenic pressure causing perturbations in the physical, chemical and biological properties of lotic ecosystems. Benthic macroinvertebrates are tools that enable rapid bioassessment. They are easy, cost-effective, quick assessment strategies to determine ecosystem health. They have been widely used in North America and Europe. However, they are hardly used in Africa. The aim of this study was to determine the impact of anthropogenic activities on the physicochemical characteristics and on the benthic macroinvertebrate communities of streams in Dschang, West Region of Cameroon, in order to determine bioindicators of the water quality. 10 sampling stations in 3 study zones were selected which were exposed to discharges from agricultural areas (Zone A), sand exploitation (Zone B) and a slaughterhouse (Zone C) respectively. Benthic macroinvertebrates, physicochemical parameters and hydrological characteristics were evaluated on a monthly basis for one year. A total of 111 benthic macro-invertebrate species distributed in 4 phyla, 7 classes, 15 orders and 45 families were recorded. In zone B, the station permanently receiving wastewater from the sandpit (B2) was the most polluted with extremely high values of turbidity, suspended solids (SS) and colour. In zone C, the station located just downstream of the slaughterhouse (C2) was also of very poor ecological quality. Zone A was the least polluted with very low values for turbidity, SS and colour and containing the most diversified macroinvertebrate community (44 taxa). The station B2 had a considerably low abundance, low values of the Shannon-Weaver diversity index and the Piélou's equitability as well as the lowest diversity with 15 taxa. The benthic macro-fauna at C2 was also less diversified with 28 taxa. Canonical Correspondence Analysis (CAC) identified 4 organic pollutant-resistant bio-indicator taxa (*Glossiphonia* sp., Chironominae, *Chironomus* sp. and *Caenis* sp.),5 inorganic pollutant-resistant taxa (*Phaon iridipennis, Zygonyx* sp. *Orectogyrus specularis, Eurymetra* sp., *Rhagovelia* sp.) and 5 taxa (*Naucoris maculatus, Naucoris* sp., *Rhyothemis* sp., *Sympecma sp., Tetrathemis* sp.) sensitive to both organic and inorganic pollution. We have identified potential macroinvertebrate bioindicator species that have the potential for biomonitoring of lotic waters in the western highlands of Cameroon and probably similar ecosystems in tropical regions.

## Fate and Effects of Metals – Regulatory and Risk Assessment Perspective

**WP026 A study of transgenerational effects on *Daphnia exilis* exposed to copper**

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Contamination of surface waters with copper is a worldwide event, usually originated by mining, agricultural, industrial, commercial, and residential activities. As a consequence, aquatic organisms are chronically exposed to sublethal concentrations of metal which can affect their fitness in different ways. One of the most sensitive groups to these stressors is the zooplankton community, particularly the cladocerans. Most ecotoxicological test guidelines are aimed to evaluate the toxic effects observed in short-time exposures or only within one generation, thus disregarding those potential detrimental effects that could appear across generations. Therefore, the objective of this study was to determine the transgenerational effects produced by copper on the American cladoceran *Daphnia exilis*, measuring demographic responses as well as responses at the macromolecular level. The acute toxic effects of copper in *D. exilis* were determined at 48 h; tested concentrations were 11, 13, 15, 17, 19 and 21 mgL$^{-1}$, at 25°C, 16:08 h photoperiod, with no food supply during assays. In the chronic toxicity studies *D. exilis* individuals were exposed to 4.9, 6.2 and 8.6 mg L$^{-1}$ copper during 21 d at 25°C, 16:08 photoperiod, feeding with 8x10$^5$ cells mL$^{-1}$ of the green microalgae *Pseudokirchneriella subcapitata*. Results demonstrated that the median lethal concentration ($LC_{50}$) was 13.45 mg L$^{-1}$. Survival and accumulated progeny in the parental generation ($F_0$) were significantly higher than those values observed in $F_1$; however, number of clutches and age to fist reproduction were not different in both generations. Concentration of lipids in *D. exilis* exposed to copper in $F_0$ and $F_1$ was not significantly higher than that measured in the control; nevertheless, carbohydrates content in $F_1$ was significantly increased respect to control. There was a decrease in the proteins content in the higher concentrations of copper in $F_0$, compared to $F_1$. Neonates size (body and total lengths, as well as body width) was significantly affected in $F_1$. In general terms, copper caused significant toxic effects on *D. exilis* mainly in the parental generation, probably because in $F_1$ the cladocerans could develop resistance mechanism to tolerate the metal (e. gr. induced synthesis of metallothioneins).

**WP027 An Evaluation of the Acute Toxicity of Lead to *Ceriodaphnia dubia* and *Hyalella azteca***

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The U.S. Environmental Protection Agency's (USEPA) current Ambient Water Quality Criteria (AWQC) for lead (Pb) was originally developed in 1984, and since then several studies have been performed that suggest water chemistry can have a significant impact on Pb bioavailability and toxicity to freshwater aquatic organisms. $EC_{50}$ data based upon total or nominal Pb concentrations may underestimate the toxicity of Pb. For a comparative assessment of two invertebrate species identified as being sensitive to Pb in the 2008 update of USEPA's National Effects Threshold database, *Ceriodaphnia dubia* and *Hyalella azteca* were selected for Pb toxicity testing in synthetic freshwater. Acute 48-hour testing with *C. dubia* and 96-hour testing with *H. azteca* was performed following standard USEPA methods. Test solutions were analyzed for total and dissolved Pb to facilitate determination of $EC_{50}$ values for Pb based on measured values. Results indicated solubility limits for Pb are encountered in lab water near the effect thresholds of both species and both species have comparable sensitivities to Pb, although *H. azteca* were slightly more sensitive. The Pb Biotic Ligand Model (BLM) was used to calculate predicted Pb effect thresholds, which were found to be comparable to the measured values generated in this study.

**WP028 Bioaccumulation of some selected heavy metals in the muscle tissue of cultured African catfish (*Clarias gariepinus*)**

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Often times, environmental scientists suggest that heavy metal contamination of aquatic ecosystem is related only to environment polluted by those metals. The present study was undertaken to investigate the bioaccumulation of Manganese (Mn), Cobalt (Co), Mercury (Hg), Asernic (As), Chronium (Cr) and Nickel (Ni) in the muscle tissue of Cultured African Catfish (*Clariasgariepinus*). A total of two (20) fish were used (male and female), each weighing an average of 390g and 310g respectively. They were cultured over a period of 6 months and fed with two feeds labeled A and B. At the 6th month, they were sacrificed and all the internal organs removed and then oven dried at initial temperature of $105^0$c and later adjust to $65^0$c until a constant weight was obtained. The samples were grinded and was digested with tri acid mixture ($HNO_3$:HCL: $H_2SO_4$). Digestion continued until the liquor become clear. The samples were analyzed using Dez Elmer Analyst 300 Atomic Spectroscopy (AAS). The result showed that Mn recorded 896.94±0.577mg/l; Co 78.10±0.577mg/l; and Ni, 78.725±0.578mg/l in feed 1 and Mn recorded 506.823±1.155mg/l; Co 32.499±0.577mg/l; Hg, 0.001±0.00001mg/l; As, 0.006±0.0000mg/l; Cr, 122.794±0.577mg/l. and Ni 56.180±0.578mg/l in feed 2. The result of the muscle tissue of male and female *C.gariepinus* shows the present of heavy metals at different concentrations and the highest concentration was observed in female recorded Ni (44.200±0.5774mg/l) and the lowest observed in male recorded in Cr (-0.007±0.0001mg/l). There was a significant difference (P>0.05) among male and female *C.gariepinus*. This study has proved the availability of Mn, Co, Hg, As, Cr and Ni in the muscle tissue of *C.gariepinus* and also in the water and feeds used in culture the fish. This metal concentration observed in muscle tissue, feeds and water was beyond the maximum permissible limit by FAO, FEPA and WHO for human consumption which their feed was the major source of contamination contrary to most view that bioaccumulation of heavy metals is related to environmentally polluted water. Based on these findings, it is recommended that proper quality control analysis should be carried out in the cultured fish to determine it safety.

**WP029 BLM-Based Ambient Water Quality Criteria and FMBs for Four Metals in Surface Waters of the Pajarito Plateau New Mexico**

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Potential aquatic life ambient water quality criteria (AWQC) for copper (Cu), lead (Pb), zinc (Zn), and aluminum (Al) based on the biotic ligand model (BLM) were generated for 48 locations representing surface waters of the Pajarito Plateau, a relatively high altitude (~2000 m above sea level) arid region west of the Rio Grande in the vicinity of Los Alamos National Laboratory (LANL), New Mexico (NM). Most waters here are ephemeral or intermittent that flow only in response to summer monsoonal thunderstorms and spring snowmelt; the few perennial waters are sourced to springs (e.g., in Bandelier National Monument) or effluent (permitted discharges). A total of 457 BLM-based instantaneous water quality criteria (IWQC) and various spatial scales of fixed monitoring benchmarks (FMBs) were generated for each metal based on LANL sample data collected largely from 2013 through 2017, with some data back to 2005. The Cu, Pb, and Zn BLM-based acute and chronic IWQC were often substantially greater than hardness-dependent IWQC based on NM water quality standards (WQS). Consequently, observed metal concentrations exceeded the BLM-based IWQC far less frequently in comparison with NM hardness-based IWQC. Cu exceeded hardness-based acute IWQC in 36% of the samples where Cu did not exceed BLM-based IWQC, i.e., using the BLM prevents 36% false positive conclusions. Further, these results suggest that twelve waters 303(d)-listed for Cu in the LANL area should be reconsidered in light of the Cu BLM-based AWQC. Similar rates of false positive exceedances of hardness-based chronic IWQC for Cu, Pb, and Zn further warrant adoption and implementation of BLM-based AWQC in state WQS. For Al, BLM-based AWQC were also generally higher than NM hardness-based AWQC, although each basis was substantially lower than EPA 2017 proposed Al AWQC, which are computed from equations using three water quality variables (pH, DOC and hardness). Compared with EPA 2017, use of NM AWQC would result in false positive Al AWQC exceedances for 11% of the unfiltered Al samples (n=457), 41% of the samples pre-filtered using a 10-µm filter (n=149), 29% using a 1-µm filter (n=34) and 44% using a 0.45-µm filter (n=457). There is currently debate and related development regarding the most appropriate sample preparation methods for Al in natural surface water samples (i.e., acidification and filtration, to restrict aluminosilicates while representing potentially toxic dissolved and precipitated forms of Al).

**WP030 Data derived from heterogeneous stock cultures linger in criteria database**

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During a literature search to derive site-specific acute and chronic zinc criteria for a stream in Colorado a potential issue with *Ceriodaphnia* studies was identified. Two studies, Mount and Norberg (1984) and Carlson and Roush (1985), which were previously included in the derivation of zinc ambient water quality criteria were found to have sourced *Ceriodaphnia reticulata* and *Ceriodaphnia dubia* from the EPA Duluth laboratory. In 1986, a review conducted by the EPA of the *Ceriodaphnia* stock cultures at the EPA Newtown Facility and Duluth laboratories found that nearly all the *Ceriodaphnia* cultures contained not one, but two species. The cultures originally identified as *C. reticulata* cultures had *C. reticulata* and *C. dubia*. The findings of this review are not insignificant, as *Ceriodaphnia* is ubiquitous with toxicity testing and many toxicity tests occurring in the mid 1980's sourced test organisms from the Newtown Facility and/or Duluth laboratories. As a result of the widespread use of *C. dubia* and *C. reticulata* in toxicity testing, many toxicity databases used to derive ambient water quality criteria include at least one toxicity test with EPA sourced organisms during which time the cultures actually contained multiple species. During our site-specific criteria development we decided to retain the Mount and Norberg (1984) and Carlson and Roush (1985) studies in the site-specific toxicity database since this was a species identification issue and not an issue related to the toxic effects. However, toxicity data for *C. dubia* and *C. reticulata* were necessarily combined and included as *Ceriodaphnia* spp. to derive the GMAV. Retention of the toxicity test results as *Ceriodaphnia* spp. for zinc rather than two separate species resulted in our site-specific zinc FAV increasing from 86.2 to 112.3 µg/L.

**WP031 Effects of water quality in Dong Nai River on nickel toxicity to *Daphnia lumholtzi***

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Vietnam is one of the fastest economic growth nations in Southeast Asia in the recent years. However, the economic growth also caused environmental consequences, especially in areas near industrial zones. Although environmental standards were recently developed, the standards are not totally relevant because of the lack of environmental toxicology data for local ecosystems. To develop a better relevant environmental standards, research on determining the toxicity of pollutants to local organisms is necessary. This study determined the influence of water quality characteristics (e.g., pH, hardness, dissolved organic carbon (DOC)) on nickel (Ni) toxicity to *Daphnia lumholtzi*. 48-h acute toxicity tests were conducted with Ni and the organisms using field collected water from Dong Nai River. Lethal effect concentrations were determined. Water quality was adjusted to represent surface water in Vietnam, ranging from very soft to hard water. In general, increasing pH, hardness, and DOC decreased Ni toxicity. When the total hardness increased from 10 to 170 mg/L as $CaCO_3$, 48-h $LC_{50}$*D. lumhotzi* increased from 87 to 1,376 µg/L, respectively. When DOC increased from 2.81 to 15.36 mg/L, 48-h $LC_{50}$ for *D. lumholtzi* increased from 96 to 216 µg/l. As the pH increased from 5 to 8.1, 48-h $LC_{50}$ for *D. lumholtzi* increased from 45 to 204 µg/L. The results will be used for setting Ni water quality standards for aquatic organisms in Vietnam.

**WP032 Reassessment of molybdenum criteria based on new toxicity data on *Tubifex tubifex***

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Currently there are no United States Environmental Protection Agency (USEPA)-derived Ambient Water Quality Criteria (AWQC) for the protection of aquatic life from molybdenum. GEI developed aquatic life molybdenum criteria for the state of New Mexico using the guidelines detailed in Stephan et al. (1985), and derived an acute criterion of 7.9 mg/L and a chronic criterion of 1.9 mg/L. Using a slightly different database, similar molybdenum criteria have also been developed for the state of Nevada. In both cases, the most sensitive species in the acute database was *Tubifex tubifex*. This *Tubifex* value ultimately drove both the acute and chronic criteria values, since the chronic criterion was derived using the acute-to-chronic ratio. During a recent review of this criteria, we determined that this 1991 *Tubifex* study was questionable and not appropriate for use in molybdenum criteria development. This has also been the conclusion in recent criteria updates conducted by EPA, as toxicity of other metals were also tested in the 1991 *Tubifex* study. A new acute *Tubifex* study on molybdenum was conducted in 2017, and the $LC_{50}$ determined by this study was two orders of magnitude higher than the previous study. As would be expected, use of this updated *Tubifex* value results a significant increase in the acute and chronic molybdenum criteria values. When the chronic criterion derived for New Mexico is updated with this *Tubifex* data, it is more in line with recent calculations based on the methodology used in Europe for development of hazard concentrations. In addition, if this 1991 *Tubifex* study was used in older EPA criteria calculations for other metals that have not been recently updated, there may be other criteria that should be updated accordingly with the new *Tubifex* value.

### WP033 Risk Assessment of Inorganic Arsenic from Dietary Intake in the United States

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Contaminated levels of arsenic (As) in the environment are extensively monitored due to its adverse global health effects that impact 150 million people in around 70 countries. The accurate risk assessment of arsenic, however, is challenging because arsenic is present in a mixture of inorganic and organic species in drinking water as well as various dietary products especially rice and chicken. Rice contains high levels of arsenic because it can effectively accumulate arsenic from soil, whereas chickens widely received arsenical drugs (roxarsone) for faster weight gain. Based on the environmental prevalence and major pathways to human receptor, we herein hypothesized that the main risks of arsenic exposure in the U.S. were from the consumption of chicken, rice and drinking water, and the risk levels depend on geographical locations and various ethnic groups with different diet patterns. This research was conducted to calculate risk using the most toxic inorganic arsenic (iAs) concentration data by the FDA and EPA along with the amount of rice and chicken consumed in U.S. household based on the USDA. Cancer risks for three different regions across the U.S. (i.e., the south, the mid and west regions) and four races in these regions (i.e., white, black, Hispanics and Asians) were estimated. The risk calculated using our compiled data was verified by an existing risk calculator (ATSDR calculator) created by the EPA. Results show that there is no immediate risk for U.S. residents across the three regions from rice and chicken. The risk levels of rice and chicken were in the range of $7\times10^{-5}$ to $9.9\times10^{-7}$ and $1.0\times10^{-6}$ to $1.0\times10^{-8}$, respectively. Based on dietary habits, black people were at the highest risk from chicken at $1.0\times10^{-6}$, whereas the Asian were at the highest risk from rice at $7.0\times10^{-5}$. However, our data reveal a significant threat from arsenic contaminated drinking water, particularly in the west region. The risk in the west was $3.66\times10^{-2}$, which is about 7 times higher than the risk in the south and mid regions. This risk level exceeds the risk permitted by the USEPA. This study demonstrates that even though the risk is manageable in rice and chicken, there is a potential threat to the U.S. population in certain regions with As-contaminated drinking water.

## Micro- and Nanoplastic Methods for Environment Media

### WP034 Microplastics in marine sediments: An assessment of current extraction and isolation methods

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Despite frequent field observations of microplastics (plastic particles < 5mm in size) there is a critical knowledge gap of their fate and effects in marine and estuarine environments. Relative to fate, much of the microplastics will ultimately accumulate in marine sediments as a result of physicochemical and biological processes. Many methods exist for the extraction and isolation of microplastics from marine sediments, but major procedural differences prevent meaningful comparison among methods. These differences may result in altered recoveries of varying polymer types, sizes, and shapes present in environmental samples. The goal of this research was to conduct a systematic assessment and intercomparison of five commonly used methods for microplastic isolation and identification in representative marine sediments. Methods were selected to reflect the range of procedures in the scientific literature. Each method was reviewed for its applicability in two sediment types (sandy and silty) as well as evaluated on their ability to recover microplastics amended into sediment samples. The microplastics chosen include a range of polymers, sizes, and shapes that are commonly identified in published field surveys. The research allows for quantification of the performance of the five methods, as well as provides initial recommendations for routine microplastic monitoring procedures in marine sediments.

### WP035 How could all that stuff be in my blank!?

*K.T. Ho, M.A. Cashman, USEPA / Atlantic Ecology Division; S.M. Russo, Oak Ridge Associated Universities / USEPA, Narragansett; R.M. Burgess, USEPA / Atlantic Ecology Division*

Awareness of microplastics in our environment, and the commitment of research to understand their fate and effects is rapidly increasing. Accurate measurement of microplastics in environmental matrices is key to understanding sources, transport, sinks, and ultimately ecological effects and human exposure. As with any environmental contaminant, using and quantifying appropriate controls and blanks are essential to accurate measurements. Obtaining and maintaining quantifiable and acceptable operational blanks with microplastics often entail more and different procedures than conventional chemists and research scientists are accustomed. Clean laboratory and forensic approaches must be followed. For example, air handling systems, particularly older ones, need to be fitted with micron level filters, and the use of synthetic material (e.g., polyester clothing) which may shed fibers should be minimalized. This presentation will detail the steps taken to obtain a microplastic "clean" space in an old (40 year) marine research laboratory. Steps taken included "deep cleaning" of all horizontal surfaces in the laboratory space, application of hepa-filter air filtration, creation of a space wrapped in cotton cloth, use of a semi-enclosed water table (or working space), installation of a laminar flow hood, replacement of a synthetic microscope cover with one made of cotton, use of cotton lab coats and cotton wipe cloths, and addition of an air ionizing bar. Through these upgrades, operational blanks with only zero to two micro-sized fibers were obtained routinely.

### WP037 A novel fluorescence-extraction assay for the quantitative analysis of nanoplastics in biological tissues

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Globally, vast amounts of plastic are produced every year. Concern has increased regarding the impacts of plastics and its degradation products on aquatic ecosystems. Plastic debris in aquatic environments are subject to an array of abiotic processes, such as sunlight, oxidants and physical stress, which cause plastics to weather and degrade, forming micro- and nano-plastics. These degradation products need careful evaluation. Currently due to analytical limitations, the options to study the uptake and transfer of nano-plastics within biological systems is limited, and the fluorescence-methods in place have issues, e.g. background autofluorescence of tissues, which can impact the interpretation of the data, and in the worst-case scenario lead to false conclusions. Hence, it is pertinent and timely to develop accurate and repeatable methods for quantifying nanopolymers, within in vivo uptake and bioaccumulation studies. To study the transfer of nano-plastics between organisms, we developed a novel fluorometric assay, to quantitatively measure the tissue burdens of fluorescently-doped nano-polystyrene (fPNP). The performance of the assay was optimized, in terms of solvent extraction, sensitivity and reproducibility. In addition, the stability of the fluorescein-label over time and susceptibility to photobleaching was addressed. The assay was validated using several relevant model organisms:algae *Chlorella vulgaris*, water flea *Daphnia magna* and three spined stickleback *Gasterosteus aculeatus*. The optimised fluorometric-assay showed the following parameters: the limit of quantification (LOQ) for PNP measurements was 0.06-0.11µg/mg (10xStd of the control S+mean), and the effects of photo bleaching on PNP dye during the experiment was minimal. The dye-extraction from biological tissues was consistent between assays with a coefficient of variation of 2.33%(CV). Greater than 96% of the dye was extracted into the organic solvent phase (supernatant) and the recovery of fluorescence following extraction from algae-tissue homogenate was 86.65±5.2%. We also derived an optimisation protocol to assess the tissue-dependent effects of any selected species, to evaluate their effects on the quantitation of fPNP. In conclusion, we show that by solvent extracting the fluorophores prior to spectroscopic analysis, the limits of detection and recovery in the presence of tissue are sufficiently sensitive to allow the application of our assay in vitro and in vivo.

**WP038 Transcriptomic and apical effects of a polyethylene microplastic on *Daphnia magna* in a 21-day chronic study**

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There is growing concern regarding the fate and effects of microplastics in the environment, particularly in aquatic systems. Microplastics are generally characterized as water-insoluble, solid polymer particles that are ≤ 5 mm in size. The assessment of the potential effects of microplastics brings several challenges when designing laboratory aquatic toxicity studies. These include: selecting an appropriate test species, determining environmentally relevant concentrations of microplastics to evaluate in the study (and the units to report these concentrations), as well as setting up the test system to be able to elucidate the factors responsible for any observed effects (i.e. influence of physical effects vs. intrinsic toxicity of the microplastic, eliminating in potential effects from non-polymer additives, etc.). In our assessment, we selected to evaluate the chronic toxicity of a polyethylene microplastic to the aquatic invertebrate, *Daphnia magna* following standardized guidance. Selecting environmentally relevant concentrations required literature searches into the monitoring data, with consideration for the usefulness and accuracy of different analytical tools for microplastic measurements. Concentrations were expressed in terms of particle number per unit volume, and a natural particle control was included in the study design to be able to interpret any physical effects from intrinsic toxicity. The density, particle size, and particle number of the natural particle control was kept similar to the polyethylene test material. Additional pre-work was also required to ensure that the polyethylene test material did not contain any additives with known toxicity, and the test solutions were evaluated for their homogeneity and stability prior to the chronic study. No significant effects on *D. magna* growth, survival, or reproduction were observed in the 21-day study in comparison to control treatments. At the end of the study, mRNA was extracted from pooled *D. magna* samples and transcriptomic responses were compared among treatment groups. This was done in order to examine more subtle sub-organismal effects at environmentally relevant concentrations, and to help discern if any effects observed in the study were due to physical interactions of the particle, or rather to intrinsic toxicity of the microplastic.

**WP039 Aquatic Toxicity Endpoints for Consideration in Assessing Microplastics Exposure Associated with Subtidal Sediments**

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Over the past several years, there has been a growing awareness of the extent of ecological impacts from exposure to microplastics in aquatic environments. Adverse effects have been documented in various levels of the aquatic food chain, including algae (reduced photosynthesis), invertebrates such as bivalves (reduced egg production and larval yield), fish (hepatic stress and neurotoxicity), and seabirds (organ damage from co-occurring contaminants leaching from ingested plastic particles). This paper will explore the existing microplastics literature for toxicity studies on marine and freshwater species, particularly biota residing in offshore or subtidal areas. The compiled toxicity data will be evaluated and compared to the toxicity testing endpoints recommended in the Inland Testing Manual and Ocean Disposal Testing Manual developed by the USEPA and U.S. Army Corps of Engineers. The assessment will consider toxicological effects from microplastics as a physical stressor, such as blockage of feeding appendages of filter feeding benthic invertebrates and accumulation in the gastrointestinal system of fish causing starvation, as well as a chemical stressor resulting in toxicity through direct exposure to chemicals contained in the plastic particles (e.g., BPA, phthalates). Indirect (dietary) exposure to persistent, bioaccumulative, and toxic chemicals (PBTs) that adsorb to the surface of the plastic particles (e.g., polychlorinated biphenyls [PCBs], polybrominated diphenyl ethers [PBDEs], dioxins, organochlorine pesticides [OCPs]) will be evaluated relative to the previously mentioned testing manuals. The ultimate goal of the study will be to assess the appropriateness of the endpoints in the testing manuals to detect toxicity and bioaccumulation associated with microplastics in sediment. A summary of the identified data gaps in the toxicological information available for microplastics for the types of aquatic and benthic communities found in offshore and subtidal environments will also be provided.

**WP040 Considerations in Selecting Indicator Species When Estimating the Environmental Hazards of Microplastics Exposure Associated with Subtidal Sediments**

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There are growing concerns related to the potential ecological risks associated with microplastics in marine waters and bottom sediments. In terms of sediment, to date, studies evaluating microplastic exposures have primarily focused on intertidal and beach marine environments; however, there are limited data focused on shallow subtidal marine environments. This paper will explore whether existing microplastics literature data for marine species are adequate to reasonably evaluate exposure to species residing in subtidal areas (e.g., kelp, eelgrass, amphipods, polychaetes, clams, sea urchins and crabs). The assessment will consider such variables as 1) the likelihood of species being exposed to microplastics via multiple exposure routes, 2) propensity of a given species to excrete microplastics, and 3) the importance of microplastics particle characteristics (e.g., size, shape, color, specific density, chemical composition) on uptake potential. In general, the ingestion pathway is considered the predominant exposure pathway, but this may not be true for some invertebrates based on particle size. For example, in one study, ingested microspheres were retained within crab body tissues for up to 14 days, while exposure to microspheres via gill transfer were retained for up to 21 days. For some species ingested microplastics can block organs by obstruction which can effect feeding. Therefore, the particle sizes of microplastics are an important consideration for indicator species selection. Other considerations include the identification of prey species more likely to accumulate microplastics and transfer them through the food chain and to what extent exposure to microplastics will change for a given type of aquatic receptor as a result of sedimentation or suspended sediments. Altered exposures from persistent organic pollutants sorbed to microplastics via ingestion by upper trophic levels, such as fish-eating birds, marine mammals, fish and invertebrates, is also important to indicator species selection. Lastly, a decision framework will be presented to guide the selection of suitable indicator species for environmental risk assessments focused on microplastics hazards associated with subtidal sediments.

### WP041 Initial survey of microplastics in bottom sediments from US waterways

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Microplastics are increasingly found in water bodies as a result of the widespread use and disposal of plastic products. The US Army Corps of Engineers (USACE) has observed microplastics (< 300 µm) in sediments recently collected from routinely dredged Federal navigation channels in New England. Despite rising evidence correlating the increase of microplastics to aquatic life toxicity, there is a lack of understanding of the magnitude of the problem in bottom sediments. While microplastics may be present in shoaled sediments routinely dredged by the USACE, it is currently unknown whether the presence of microplastics may be harmful to aquatic species occurring in ports, harbors, and navigation channels. The objectives of this research were to: 1) perform a literature review to better understand the current science on the quantity and quality of microplastics in bottom sediments from the US and elsewhere around the world; 2) quantify microplastic content in over 20 dredged sediment samples collected from waterways across the U.S. via particle scanning flow cytometry and Fourier Transform Infrared Spectroscopy (FTIR). The results of the literature review indicate that microplastics are ubiquitous in bottom sediments in the U.S. as well as from other countries. Additionally, the majority of studies investigated particle sizes above 300 mm, while our study addresses the methodological challenges and ecological relevance specifically of small-scale microplastics (mm). Completing these objectives will assist in improving our understanding of microplastics in bottom sediments which are of growing importance to the USACE. Understanding the nature, extent, and potential impacts of microplastics in dredged sediment will allow USACE Districts to apply this knowledge to dredging projects where microplastics are of concern.

### WP042 Temporal variability of microplastic abundance in intertidal sediments: Implications for sampling frequency

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The occurrence, distribution, and composition of microplastics (< 5 mm) are well documented on shorelines worldwide. However, high variability in microplastic abundance is often observed within and among studies. The majority of microplastic surveys to date consist of single sampling events that do not consider temporal variability as a potential confounding factor in the interpretation of their results. Therefore, these microplastic surveys may not accurately represent microplastic abundance, distribution, and composition. Here, we investigate the small-scale temporal and spatial variability in the abundance and distribution of microplastics in the intertidal zone of a sandy beach in the Charleston Harbor estuary, South Carolina. Intertidal sediment was collected from the low intertidal zone (n=3) and high intertidal zone (n=3) at low tide over 17 days (12 sampling events; total n=72). Over the course of the study, microplastic abundance ranged from 44-912 microplastics/$m^2$. Microplastic abundance differed significantly among sampling events (p=0.00025). In addition, microplastic abundance differed significantly among some consecutive tidal cycles (p=0.007). Over the course of the study, microplastic abundance did not differ significantly between the low intertidal zone and the high intertidal zone (p=0.76). These results indicate that there is significant temporal variability of microplastic abundance in intertidal sediments of the Charleston Harbor estuary. Accordingly, future microplastic surveys need to include a temporal component in their study design to more accurately quantify microplastic abundance.

### WP043 Microplastics in a suburban stream

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Sources of microplastic particles to rivers are not understood very well. The concentration of microparticles in the Chesapeake Bay and some of its tributaries have been measured, but their sources have not been identified. To find out if the surface runoff and the WWPT discharge are sources Four Mile Run, a small suburban stream that empties into the Potomac river near Washington, DC, and the discharge from the Arlington Virginia Water Pollution Control Plant and were sampled. Particles were collected by securing 25 sq. cm of 0.3 mm polyester mesh screen to the end of a 500 mL funnel and pouring 10L of water through. Three samples were collected at each of four locations in the stream and the WWTP discharge. The mesh with particles was removed from the funnel, labeled, wrapped in aluminum foil and stored in zip lock bags for transport out of the field. Each sample was placed a glass petri dish and immersed in 35% hydrogen peroxide for 24 to 48 hours to remove natural organic material. The digested samples were examined under 40x magnification, the particles counted, and size and appearance recorded. Microplastic particles were found in all samples, at average concentrations of 0.30-0.55 particle per liter. Most appeared to be polyester fibers. Others appear to be rounded polyethylene other polymer, based on visual inspection. Based on the average stream flow rate of 50 million liters per day Four Mile Run delivers 25 million microparticles per day to the Potomac River. The sewage treatment plant adds 30-40 million particles per day. These data suggest that the stream and WWTP are significant contributors to microparticles to the Potomac River.

### WP044 Rain Gardens Mitigate Microplastics Entering Aquatic Habitats

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Urban runoff is suspected to be an important pathway for microplastic to enter San Francisco Bay, but there are very few studies that report microplastic concentrations in stormwater. A bioretention rain garden draining an urban watershed area composed of roads with heavy car and foot traffic and high density residential and commercial land use was monitored through three separate storm events in 2017. Composite influent and effluent samples were collected through the hydrograph of the storm. Particle samples were separated by density separation, individually enumerated and categorized under a dissecting microscope, polymer types were identified by Raman spectroscopy. Results include particle concentrations in stormwater, as well as the breakdown of particle concentrations by particle morphology and polymer type. Comparison of influent and effluent concentrations show high particle removal efficiencies. Additionally, field blanks collected provided quality assurance for the data. Due to the limited amount of data on microplastic in stormwater, this information is a significant contribution to the field of microplastic research.

**WP045 Influence of particle size on prospectively modeled environmental concentrations of microplastics in the Sandusky River watershed**

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The presence of nano- and microplastics (MPs; particles < 5 mm) in the aquatic environment is a topic of increasing discussion and research. Although measurement and monitoring data are indispensable, there is a need to prospectively estimate concentrations to enable forward-looking assessments and to guide analysis of retrospective ecological analyses. For traditional chemicals, fate and exposure models have been proven to be very helpful and are widely used. However, to date few models exist that simulate the transport and fate of MPs in freshwater systems. This presentation presents simulations of the transport and fate of various-sized MPs emitted from wastewater treatment plants into freshwater riverine systems, and tracks concentrations moving downstream from headwater into Lake Erie. We linked the NanoDUFLOW model (a detailed MP aggregation-sedimentation model integrated in a hydrological and particle transport model) with iSTREEM® (developed to estimate chemical concentration distributions for all rivers receiving WWTP discharges in the US) for a range of particle sizes. This combines the mechanistic realism of NanoDUFLOW, accounting for formation and settling of heteroaggregates, with the US well-established iSTREEM implementation. Depth dependent in-stream first order removal rate constants simulated with NanoDUFLOW were combined with standard iSTREEM output which simulated the emission, transport and water column concentrations of different MP sizes. We modeled floating as well as non-buoyant MP, for sizes ranging from 100 nm to 1000 µm. We also modeled a combined mixture of particle sizes based on effluent measurements from Mason et al (2016). Simulations were spatially explicit with MP concentrations being modeled for the Sandusky River watershed in Ohio containing over 300 miles of river downstream of 20 WWTPs. Modelling results show the effects of population density, MP size and environmental conditions on riverine concentrations and export to Lake Erie. Buoyant as well as the smallest non-buoyant MP fractions can be transported over long distances, reaching receiving waters such as the Great Lakes. In contrast, larger non-buoyant MPs settle more locally in the vicinity of the WWTPs.

## Incorporation of Sustainability into Undergraduate Curricula in Science and Engineering

**WP046 Community Based Learning in Teaching Sustainability Engineering**

*A. Hicks, University of Wisconsin, Madison / Civil and Environmental Engineering*

The three paradigm of sustainability: environmental, economic, and societal, are challenging for students to fully grasp and apply in an engineering context. Community based learning (CBL) is an approach to contextualize the paradigm, and to allow students to evaluate the sustainability of a particular system. Utilizing CBL also served as an avenue for students to better understand the uncertainties present in real-world engineering situations, and to engage with stakeholders in the broader community. CBL was introduced to the "Environmental Sustainability Engineering" course at the University of Wisconsin-Madison in the Fall 2016 semester (and was continued in the Fall 2017 and 2018 semesters) . Students chose one of three community based projects, including a potential solar panel installation for a low income housing community, salting practice changes as a method of reducing chloride run off to a local lake, and the egg production for a farm to table restaurant. Students evaluated the systems through the lens of industrial ecology, and utilizing the three paradigm to make their recommendations. Within this, tools such as cost/benefit analysis, payback period, economic input output life cycle assessment, and societal changes were utilized to by the students. The students were assessed at the beginning and end of the semester to gauge their knowledge of topics to be covered in the course, and again at the end of the semester. These are also compared to the same knowledge probe which was utilized the previous time the course was offered without the CBL component, to better understand knowledge gains, and the influence of incorporating a CBL component to the course. The results of the knowledge probe will be presented, along with challenges encountered (and how to overcome them) with the CBL aspect of the course.

**WP047 Leveraging scientific studies popularized by social and news media outlets to engage students of various educational backgrounds**

*E. Medlock Kakaley, USEPA / UNC Cooperative / Dept of Biological Sciences / Environmental and Molecular Toxicology Program; I. Jaspers, Curriculum in Toxicology / University of North Carolina at Chapel Hill*

Everyday we are bombarded with half-true sound bites and inflammatory news statements about the world we live in, more specifically the latest scientific findings. In this introductory toxicology course students read, discussed and presented recent news stories and corresponding cited peer-reviewed literature. Through experiential learning, students interpreted the true meaning of scientific findings and acquired applicable presentation and peer-review skills as well as foundational toxicology concepts. Students were polled pre- and post- semester with an online survey tool for self-assessed knowledge of toxicology and expected/actual acquired skills. All assigned materials for each of five course modules were provided electronically through the university education platform. Introductory information was presented by local field experts for 4 of 5 modules. Students presented their assessment of scientific interpretations made by both researchers and reporters/journalists through a Powerpoint® presentations or in a discussion-based format. Fundamentals were covered on an as-needed basis during class discussion. The academic level and program was highly mixed in the small class size of 6, 5 of which had never enrolled in a toxicology course prior to Spring 2018. However, the range, recent media coverage, and expert-led introduction of each module made topics more accessible, allowing for ample participation from all students. In fact, the range of self-identified "knowledge of field" and "knowledge of current events in the field" increased from "little prior knowledge" to "somewhat knowledgeable" in the pre-course survey to "somewhat knowledgeable" to "very knowledgeable" after course completion. Students over-whelmingly reported favoring the teaching modules, and despite reports of instructor's sound knowledge of the field and willingness to discuss fundamentals some reported lack of intentional direction in course discussion. Ultimately, 1 of 2 admitted/undecided students chose the toxicology track of study. Therefore, this course will again will be offered in Fall 2018 to all students with interest in the field of study. Module introductions will be instructor led, or instructor will more clearly guide content of expert-led introductions, to better emphasize field fundamentals. Thus allowing ample application of fundamentals during student-led news/article assessment and creating more intentionality during discussion.

**WP048 Planetary boundaries in STEM education towards a sustainable future: Integrating sustainability into the undergraduate curriculum**

*J. Blatti, Pasadena City College / Chemistry*

This talk will discuss various approaches to incorporate sustainability into the undergraduate curriculum, including undergraduate research in sustainability, curriculum development in sustainability, the development of interdisciplinary, cutting edge labs that emphasize the principles of Green Chemistry, and sustainability outreach and education to underrepresented populations, which includes a campaign to promote diversity in the STEM workforce. Under the theme of Sustainability, topics such as renewable energy, climate change, biopolymers and sustainable materials are explored. Research in these areas is translated to outreach lessons to bring awareness of sustainability and Planetary Boundaries (PB) to underrepresented communities and to inspire the next generation of scientists to use their skills to solve the environmental challenges of our time. As part of the Sustainability Science and Engineering course in the Winter of 2017 at Caltech's Resnick Sustainability Institute, we evolved a project to use the framework of PB as a platform to discuss how each aspect of sustainability science relates to students' lives, as oftentimes students do not see the connection between these big ideas and their actual lives. We use hands-on laboratory experiments, such as the synthesis of biodiesel from algae oil, making paints from sustainable materials, and DNA nanotechnology as a way to drive in depth discussion of these topics. Although there is a strong desire from sustainability educators to disseminate lessons and make a broad impact, efforts in the area of sustainability education remain localized. Promoting diversity in the STEM workforce is an opportunity to make a broad impact in sustainability education and sustainability science. Lastly, this talk will discuss ways to emphasize creative problem solving in science as part of the scientific method, which includes art-science intersections as a means for students to develop critical thinking skills so that they can think 'outside of the box' and solve imminent environmental challenges of our time.

## Integrating Cooperative Federalism into Environmental Research and Assessments

### WP049 State-Federal Research Collaboration through the National Estuary Program

*G. Cicchetti, USEPA / Atlantic Ecology Division / National Health / Environmental Effects Research Laboratory / Office of Research and Development; W.S. Fisher, USEPA / Office of Research and Development / National Health and Environmental Effects Research Laboratory*

Estuaries are among the most productive ecosystems in the world, providing unique habitat for freshwater and marine species as well as valuable social and economic benefits. The wealth of ecosystem goods and services from estuaries has led to growth and development of human communities in adjacent areas and a concomitant increase in human activities that degrade habitat and water quality. Management for sustainable estuaries necessarily balances environmental concerns with community social and economic values. The non-regulatory National Estuary Program (NEP), instituted by the Clean Water Act and supported through EPA's Office of Water, provides opportunities for stakeholders to manage 'nationally significant' estuaries. Each of the 28 estuaries currently in the NEP engages a diverse community of stakeholders, ensuring that local economic and social values are incorporated into estuary management plans. The real-world decisions faced by NEP managers provide fertile ground for applied research on estuarine water quality and aquatic life resources. The coastal ecology Divisions of EPA's Office of Research and Development, National Health and Environmental Effects Research Laboratory, are engaged in water quality and aquatic life research and have worked closely with NEP managers on multiple projects across the United States. Several of these collaborative efforts are highlighted with the intent of fostering even greater focus and participation.

### WP050 TechTracker Tool Documents Cooperative Federalism at the USEPA

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With publication of the FY 2018-2022 EPA Strategic Plan, the U.S. Environmental Protection Agency (USEPA) endorsed cooperative federalism to improve environmental protection through shared governance and enhanced collaboration by the USEPA with state, tribal, local, and federal partners. This evolving reliance on collaboration across government at all levels is supported by the EPA Office of Research and Development (ORD). Increasingly, ORD is seeking input from USEPA partners and stakeholders to identify pressing environmental problems and provide support for science-based solutions. Herein, we report on the topics and demand for ORD scientific and technical expertise based on data from TechTracker, a new tool developed to document the regulatory, programmatic, and scientific support that ORD provides to partners and stakeholders. The tool documents the hours, topics, and users of ORD's environmental expertise. Analysis of more than 34,000 hours of consultations recorded by ORD scientists since the beginning of 2018 fiscal year shows the majority of the more than 2500 requests came from within the agency; that is, from the ten USEPA regions, the program offices and intramural research Laboratories and Centers. All 50 states, plus U.S. territories, >50 federal agencies, a dozen countries, 26 tribes, 54 cities and communities accounted for an additional 25% of the requests. Topics generating the most requests aligned well with the core USEPA mission—to protect human health and the environment. ORD respondents most frequently chose, in order, (1) ecology/environment, (2) human health, and (3) chemicals as most descriptive of their queries. Scientists were usually asked (55% of requests) to provide technical information or to review technical documents. We also tracked interest in topics of hazard characterization, microbiology, regulation and other policy actions. Queries were infrequently categorized as sustainability, social sciences, or economics/benefit analysis. We designed TechTracker as an internal tool to understand demand for technical expertise among our partners and stakeholders as well as to foresee potential needs for expertise and areas of emerging interest. Results to date indicate the importance of EPA outreach to partners and stakeholders as cooperative federalism rises on the federal agenda.

### WP051 Application of the Ecotoxicology Knowledgebase (ECOTOX) to support environmental research and risk assessment

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The ECOTOX Knowledgebase (http://cfpub.epa.gov/ecotox/) is a nationally and internationally recognized source of curated single-chemical toxicity data for aquatic life, terrestrial plants, and wildlife. This publicly available database currently includes data on more than 11,000 chemicals from over 47,000 references. ECOTOX data are used for all ecological risk assessments supporting pesticide registrations and re-registrations, all ambient water quality criteria for chemicals published since 1985, site-specific water quality criteria (by EPA Regions, States, and Tribes), and assessments used in emergency response. A significantly enhanced interface (v5.0) of the ECOTOX Knowledgebase was released in 2018. Advances include the integration of improved and computationally automated literature search strategies for data curation, and inclusion of more mechanistic (e.g., genetic, enzymatic) and pathway-based (e.g., hormonal, cellular) data to better align with the evolution of toxicity testing. This newly released ECOTOX user interface has enhanced functionality for searching and exploring data, with interactive data visualization capabilities. Further, links to other EPA tools (e.g., CompTox Chemistry Dashboard) and databases were integrated, laying the foundation for future interoperability. Presented here are three case studies demonstrating how ECOTOX v5.0 has improved to support environmental research and risk assessment. The case studies focus on: 1) linking detections and concentrations of environmental contaminants to effects data; 2) identifying empirical data to support and expand the development of adverse outcome pathways; and 3) gathering data for development of toxicity reference values. In these examples, the new ECOTOX *Explore* options were used to evaluate the scope of data availability, observe potential trends across chemicals or biological species, and develop testable hypotheses, thus demonstrating how improved features allow for rapid acquisition and analysis with the most relevant available toxicity data. With the new and improved ECOTOX platform, researchers and risk assessors can explore, visualize, identify, and download curated toxicity effects data that best match exposure scenarios, species of interest, and most appropriate assessment and measurement endpoints for developing or addressing focused research questions and decision-making. This abstract does not necessarily reflect USEPA policy.

**WP052 Environmental Toxicology Data Quality Evaluation, Extraction and Data Integration Under TSCA**

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In 2016, the Frank R. Lautenberg Chemical Safety for the 21st Century Act amended the Toxic Substances Control Act (TSCA), the nation's primary chemicals management law. To meet the scientific standards under TSCA, the Office of Pollution Prevention and Toxics (OPPT) is conducting systematic review to evaluate the ecotoxicology data for the first 10 chemicals undergoing risk evaluation. The new process, already in progress, included a first step in which a search strategy was developed and used to gather environmental toxicity information. The titles and abstracts for citations identified in the search were screened using ECOTOXicology knowledgebase (ECOTOX) criteria to parse them into "on-topic" and "off-topic" bins. The "on-topic" references were then subject to a full-text screening using additional, more specific ECOTOX criteria to evaluate study relevance and determine which citations should move to the next step, data extraction. Information gathered from authoritative grey literature also underwent the full-text screen and data extraction steps. EPA developed data quality evaluation (DQE) criteria under TSCA, based on a combination of EPA's ECOTOX criteria and the Criteria for Reporting and Evaluating ecotoxicity Data (CRED). EPA will evaluate each study using the DQE criteria to determine overall study confidence. The data resulting from this systematic review analysis will serve as the basis for developing a hazard characterization and will be integrated into the risk evaluation for each of the 10 chemicals.

## In Situ Treatment of Contaminated Sediments – What Are the Remaining Barriers?

**WP053 In situ activated carbon (AC) amendment to limit bio-uptake from PCB contaminated sediment – A mesocosm study with three organisms**

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In situ AC amendment (4.3% by dry weight homogenized with sediment) was investigated in an experimental mesocosm containing PCB contaminated sediment from New Bedford Harbor (New Bedford, MA, USA). AC was applied by homogenization with sediment prior to the start of the experiment. This may be feasible in the field where dredging is performed. In other cases, AC would be applied as a cap (secured with a sand or armor layer to prevent degradation) and would become homogenized over time due to bioturbation and other processes. Experimental conditions are likely representative of field conditions 10 ± 5 years after application. As this is not a practical experimental time frame, homogenization of AC and sediment allows simulation of long term conditions. Efficacy of AC amendment was evaluated by measuring freely dissolved porewater concentrations ($C_{free}$) and lipid normalized tissue concentrations ($C_{lipid}$), compared to unamended, control mesocosms. Three organisms of varying levels of sediment interaction were tested. $C_{free}$ were deduced by low density polyethylene (LDPE) passive sampling. $C_{lipid}$ were deduced by homogenization and extraction of organism tissues. Overall, decreases in toxicity equivalents were achieved with AC amendment. PCB congeners ranging from 2 to 9 chlorines (Cl) were detected in organism tissue and 2 to 8 Cl in sediment porewater. Reductions in $C_{lipid}$ were observed with AC amendment for congeners up to 6 Cl and in $C_{free}$ up to 7 Cl. Increases in $C_{lipid}$ were observed across all congeners of 7 – 9 Cl and minor increases in $C_{free}$ for one 6 Cl and two 8 Cl congeners indicating mobilization of larger congeners in the presence of the AC amendment. In order to determine whether overall toxicity equivalents were decreased by AC amendment, analysis of the dioxin-like (co-planar) congeners was performed. The sums of $C_{lipid}$ and $C_{free}$ for the dioxin-like PCBs decreased by an order of magnitude from 3550 to 361 µg PCB/kg lipid and from 4.73 to 0.121 ng PCB/$L_{Water}$ respectively, indicating a decrease in expected toxicity in AC amended sediments even though increases in the $C_{lipid}$ and $C_{free}$ of some individual congeners occurred.

**WP054 Novel In-situ Toxicity Assessment of Sediment Cover Effectiveness in Deep Water**

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A former mining site has been the subject of intensive restoration for the past few years, with significant focus on disconnecting mine spoils from groundwater and managing the quantity and quality of runoff. An investigation was conducted to compare the efficacy of selected cover materials for decreasing Zn dissolution during periods when the hypolimnion is anoxic and acidic (pH=5.5). Cover materials were selected based on results from laboratory batch testing and included AquaBlok, limestone, and limestone + bonechar. Experimental field tests implemented novel methodologies, using Limnocorrals (LC) to isolate water columns above various cover treatments, simulating lake-mesocosms. Simultaneous in-situ and ex-situ toxicity tests were conducted using *Daphnia magna*, *Hyalella azteca*, and *Chironomus dilutus*. Test organisms were protected from temperature shock by pre-acclimating over 24 hrs and then deploying the test chambers in a Toxicity Assessment Container System (TACS), which protected the organisms from warm surface waters until reaching the bottom sediments and colder water. Test organisms were exposed to surficial sediments and overlying water in the reference (no cover) LC and in the two LCs containing surficial sediments plus cover materials. Ex-situ testing was conducted in waters and/or sediment cores collected from the bottom of each LC, and these tests were done at the same temperature as the in-situ TACS exposures (15 to 19 C, depending on deployment period). Laboratory tests involved a series of acute toxicity tests and water chemistry sampling conducted in core microcosms created from site-collected sediment. Results from in-situ testing demonstrated the usefulness of the TACS and provided similar results to the ex-situ testing. Overall, there were no differences in biological responses/endpoints between treatments involving sediment cover materials, and this was true in both the field and laboratory tests. This was likely due to the fact that dissolved Zn concentrations in the surface water were below-threshold levels in all treatments. All treatments (AquaBlok, limestone, and limestone + bonechar) successfully reduced Zn release from the sediment, although some may be less effective under certain hydrologic conditions. Results provided for more effective decision-making, with reduced uncertainty, than standard laboratory and chemistry-only approaches. Follow up testing will examine zinc release delay via dissolution rates.

**WP055 PCB Tissue Concentrations and Benthic Community Impacts at a Carbon Amendment Pilot Study in the Intertidal and Subtidal Zone of San Francisco Bay**

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Historical site activities at the Hunters Point Naval Shipyard (HPNS) and other off-base locations in South San Francisco Bay resulted in the release of chemicals, including PCBs, to offshore sediments. To inform remedy selection at HPNS, activated carbon (AC) amendments alternatives were evaluated in a pilot treatability study. Two 0.4 acre plots extending from the intertidal to the subtidal zone treated with either AquaGate + PAC™ or SediMite™ were assessed for the potential to reduce ecological risks associated with PCB-contaminated sediment. Previous treatability studies indicated that AC may be effective at reducing the bioavailability of PCBs to the bent-nose clams (*M. nasuta*) in shallow intertidal sediments when aided by mechanical mixing. This study assessed the effectiveness of AC placements without mechanical mixing in deeper water that is more representative of conditions where full-scale remediation is expected. Tissue bioaccumulation, benthic invertebrate community composition, and chemical analyses were measured as indicators of remedy effectiveness. Comparisons were made between baseline, reference and post-amendment conditions (8, 14, 20, and 26 months post-placement). PCB tissue concentrations in *Macoma* sp. were measured in situ and *ex situ* (bench-top) after 28-day exposures. Developing field exposure chambers that allowed sediments to infiltrate the chambers and expose clams upon deployment and retrieve the sediment and exposed organisms for chemical analyses was a challenge. Modifying a chamber design used in previous studies by Luthy et al. (2009) proved successful. Test organisms were another challenge. Tissue bioaccumulation was planned to be conducted with *M. nasuta* but instead, initial measurements were made with *M. secta* (white sand clam) collected at a nearby reference location where *M. nasuta* had been previously found. The species have a similar appearance and life histories but *M. secta* had low survival in the field (< 20%), lab exposures (< 60%), and lab controls (10%). Additional field pilot testing led to the use *M. nasuta* from a supplier for post-amendment monitoring. PCB tissue concentrations were reduced by approximately 90% in Plot 1 and 80% in Plot 2 amendment areas after 26-months post-placement. Benthic invertebrate communities in test plots showed greater diversity at 26-months post placement compared to other monitoring events, indicating that the AC treatments had no long-term negative impacts on community health.

## Environment Exposure to Microplastics and Affiliated Toxic Chemicals

**WP056 A novel technique to qualify and quantify fine microplastics in surface water by coagulation and μFTIR microscopy**

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The occurrence of Microplastics (MPs) is of great concern in aquatic environment, especially ocean. Many current studies evaluate MPs ranged from 100 μm to 5 mm. However, MPs used in personal care products and other industrial processes are reported to be smaller according to previous reports. Because MPs are very important substances to current economic activities, new materials for MPs will be needed such as cellulose. But before the alternative of the materials, contamination levels of these fine MPs in aquatic environment should be revealed. This study shows a new analytical technique to qualify and quantify these MPs in surface water. One of the new applied techniques is coagulation by Poly Aluminium chloride after density separation process. This new process can easily decrease matrices such as plant debris, algae, fine soils and other nonplastic particles on a membrane. Recovery tests were conducted using surface water contained known amount of three different size MPs and fiber type MPs. The results showed very good recovery rates of all types of MPs. After density separation, μFTIR microscopy with mapping image system and multiregression analysis. This system enables automated multi-point mapping, line mapping and IR Imaging analyses of a microscopic area with a manual sample stage and a single element detector. Though it is very difficult to quantify and qualify MPs of less than 100μm diameter by a stereoscopic microscope, this developed method can easily measure fine MPs on a membrane. The developed method can reveal characteristics of fine MPs in sewage water, sewage treated water, surface water with high concentration of suspended solids in order to evaluate their occurrence in aquatic environments and their ecological risk assessment.

**WP057 Biosolids inhibit the bioavailability and bioaccumulation of triclosan and triclocarban from microplastic to plants and earthworms**

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Numerous varieties of microplastics enter agroecosystems from various routes and have potential for transporting chemical pollutants to organisms. Biosolid, rich in organic carbon, agronomic applications can increase the sorption of certain pollutants in soil, decreasing their bioavailability and bioaccumulation. This study tested the hypothesis that microplastic moves typical personal care products, triclosan and triclocarban, to the radish and earthworm, and the relative bioaccumulation of personal care products decreases with increasing biosolids amendment. Accumulation of triclosan and triclocarban was observed in roots of radish and earthworms grown in soils with 0.5%, 2% and 5% microplastics spiking with 200 mg $kg^{-1}$ pollutants. Addition of biosolids significantly decreased bioaccumulation of triclosan and triclocarban in roots of radish and earthworms. Using a thin-film passive sampler, polymethylmethacrylate film, we found that the availabilities of triclosan and triclocarban greatly decreased in biosolids-amended soils. The study provided the evidence that agronomic biosolid applications inhibit the bioavailability and bioaccumulation of personal care products from microplastic to plants and earthworms.

**WP058 Chemical analysis of ingested plastics and associated organic pollutants in wild-caught black sea bass, *Centropristis striata***

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The accumulation of microplastics in oceans, tiny granules of plastic < 5mm in diameter, is one of the major environmental concerns of our age. Due to their large specific surface area and hydrophobic properties, microplastics can absorb numerous organic pollutants. Intake of microplastics by marine organisms may therefore result in introducing toxicants to the organism itself and to the food chain, possibly leading to their bioaccumulation. Though a number of marine organisms have been found to ingest microplastics, few studies have been performed on commercial fishery species such as black sea bass (*Centropristis striata*). Here we have studied plastic ingestion by wild-caught black sea bass, an important fishery off the East coast of the United States. Our preliminary results showed that out of 102 fish sampled off the coast of North Carolina (from Beaufort, NC and Oak Island, NC), only 4 contained macroplastics. Work is in progress to digest samples in potassium hydroxide, followed by a series of filtration steps to isolate any microplastics or microfibers. We are using several spectroscopic and chromatographic techniques to characterize already-detected plastics and to identify the pollutants. Attenuated total reflection in conjunction with Fourier transform infrared spectroscopy (FTIR-ATR), and Raman spectroscopy were used to identify types of plastics. Using these techniques, we have already confirmed that one of the ingested plastics was poly(ethylene terephthalate) (PET). The identification of pollutants associated with the plastics was achieved by solvent extraction and subsequent gas chromatography-mass spectrometry (CG/MS) analysis. Tissue analysis will also be performed to understand the leaching of various chemicals and associated pollutants from the plastics. The extracts will be purified using Agilent bond elute Enhanced matrix removal (EMR-Lipid), a highly efficient method to remove lipids without impacting analyte recovery. Purified extracts will be analyzed with GC/MS-MRM (multiple reaction monitoring) method, a highly sensitive MS technique to selectively quantitate compounds from a complex mixture. We anticipate that our results from the analytical studies will contribute to the current knowledge on the plastic pollution in *C. striat*a and will inform both fishery management and potential concerns regarding human consumption of black sea bass that have ingested plastics.

**WP059 Enhancement of microplastics on PCB 126 accumulation in *Daphnia magna***

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The abundance of plastics in bodies of water has exponentially increased, which has catalyzed researchers to explore the consequences that arise from this trend. Although there have been discoveries published, none of them have reported the potential effect of plastics on polychlorinated biphenyls (PCBs) accumulation in zooplankton-a primary food source of many higher trophic organisms in the aquatic ecosystem. The primary goal of this study is to determine if the adsorption of PCB-126 on microplastic enhanced the accumulation of this compound in *Daphnia magna* and biological performance (i.e., growth, reproduction) of the organisms. *Daphnia magna* was exposed to PCBs in water with the presence of microplastic or no plastic for 21 days. Results showed that *D. magna* consumed significant number of microplastics. PCB-126 was adsorbed on microplastics and carried into the organism's gut and resulted in higher accumulation of PCB-126 in the body of *D. magna*. While concentration of PCB-126 was not detected in control *D. magna*, exposed *D. magna* with the presence of microplastics had a body PCB-126 concentration of 30.28 mg/kg wet weight, which was significantly higher than the body PCB-126 concentration of exposed *D. magna* with no microplastics (17.39 mg/kg wet weight). Results of biological measurements showed that although there was no significant difference in the performance of exposed organisms versus the control organisms, *D. magna* appeared to perform poorer in water with the presence of microplastics and PCBs than in the control. Results of the present study poses a potential risk of plastic and PCBs transfer over to higher trophic organisms in the food chain.

**WP060 Field measurements to quantify microplastics in the San Francisco Bay and adjacent National Marine Sanctuaries**

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Limited study of microplastics in the San Francisco Bay surface water suggests that Bay concentrations may be elevated in comparison to other North American locations. In addition, microplastics have been identified in fish from the Bay and the adjacent coast, indicating uptake of microplastic into the food web. In this study, microplastics were collected from the 16 sites in the San Francisco Bay and 11 sites in the Cordell Bank, Greater Farallones and Monterey Bay National Marine Sanctuaries using 20 micron pump filters and Manta trawls to characterize plastics less than 5 mm in size. The efficacy of pump vs. Manta trawl samples will be discussed. Microplastics were identified and quantified using optimized analytical methods. Discussion of sample results and understanding of the predominant polymers in microplastics and microfibers present in the Bay and sanctuaries will help to support identification of microplastic sources and to model the transport of these particles from the Bay to the surrounding ocean environment.

**WP061 Health hazard of drinking potable water stored in polythene sachet after exposure to direct sunlight**

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Packaging materials manufactured from polythene, its derivatives and other additives such as antioxidants, stabilizers, plasticizers, lubricants, antimicrobials, anti-static and anti-blocking agents, "slips," or heat resistance agents, are highly used in Nigeria for food and water. Under some environmental conditions such as heat, sunlight or UV radiation, these materials may undergo photolytic, photooxidative, and thermooxidative reactions which might produce toxic substances. If such compounds are produced and their concentrations surpassed a specified limit, quality and safety of the packaged content is compromised and this may have a serious implication for human health. In other to verify the above, eighteen (18) sachet water samples collected from three different factories (labelled A, B and C) in Nsukka, Enugu State were grouped in fives (5) and each of the three brands kept under the sun (UV) for one, two, three, four or five days. At the end of each exposure period, the sachet-water samples were collected and transferred into sterilized 200 ml Amber bottles. The samples which were not exposed were kept as control. The exposures were in triplicate. Degradation products for each exposure period were assayed using Gas Chromatography-Mass Spectroscopy with purge and trap (Agilent Technologies 7890A and 5975N mass selective detector). Some of the results obtained for sachet water from Factory A after one day exposure showed methylene chloride (16.82%), acetic acid (5.21%), octamethyl radical of cyclotetrasiloxane (5.63%) etc. Also at day 3 of exposure toluene (19.7%), 1-Dodecene, (Z) radical of 2-Dodecene and 1-Undecanol (14.3%), Xylene and 1, 3-dimethyl radical of Benzene (0.7%) etc were obtained. The same trends of results were obtained at the $4^{th}$ and $5^{th}$ day of exposure. Results for sachet water samples not exposed to sunlight (control sample) showed no trace of degraded components of polythene. Therefore, the presence of degraded components of polythene in sample from day 1 to 5 must have been due to photo-oxidation (sunlight exposure). The same trend followed for sachet water samples from Factory B and C. Some of these compounds have been confirmed by U.S. environmental protection agency (EPA) as a group c and group 2b possible human carcinogen and are widely acknowledged to have reproductive and developmental consequences as well as being possible human carcinogen.

**WP062 Identification of Microplastics Sized 3-500µm in Highly Urbanized Aquatic Environments**

*K.J. Wiggin, E.B. Holland, California State University, Long Beach / Biological Sciences*

Plastic production has drastically increased over the last 60 years, and consequently so has marine plastic pollution. Research demonstrates a vast prevalence of smaller plastic particles in aquatic environments, known as microplastics (MP;< 5mm). Common sampling protocols only sample plastics greater than 333µm, a common plankton net mesh size. To address this data gap, the presence of MP sized 3-500µm were assessed in Long Beach Harbor, the San Gabriel River, and the Los Angeles River (CA;USA), three areas with highly urbanized surroundings. Prior to sampling, the efficiency and accuracy of extraction and identification protocols utilized in previous MP detection studies were evaluated. For extraction efficiency, three commonly used digestion protocols were evaluated, including wet peroxide oxidation, enzymatic digestion (Cellulase), and hydrogen peroxide. For identification, the hydrophobic dye Nile Red was evaluated for its ability to dye plastic particles and organic materials in order to establish its efficacy as a secondary detection step. Digestion trials demonstrated that a 15% hydrogen peroxide exposure was highly efficient at digesting organic material, including natural fibers, and the entire protocol minimized contamination, degradation and loss of plastic particles. Additionally, Nile Red was found to exclusively dye plastic particles, and not organic materials. These methods were applied to 20L surface water grab samples from the field where prior to digestion, particles were size fractionated through sieves of various pore sizes. MP was then quantified and categorized under 40-200x magnification. Furthermore, 10% of MP samples were subject to Nile Red for secondary detection. This project will help establish a standard MP sampling, extraction, and identification protocol for sizes below 500µm and help to fully understand the prevalence of these smaller sized MP.

**WP063 Isolation and analysis of microplastic-associated additives using a previously described density separation unit**

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Microplastic particles are ubiquitous and heterogenous environmental pollutants. Vast differences in size, shape, and plastic type have been observed in sediment samples ranging from beaches to the deep sea. Each polymer type houses varying chemical additives and dyes to enhance the functionality and coloring of the material. Common polymer additives, such as nonylphenol and azo dyes, are of environmental concern due to their role as endocrine disrupters and suspected mutagens. Many studies have attempted to achieve efficient and thorough isolation of microplastics from sediment via floatation, density separation, and constructed separation units. However, few strategies have focused on isolation and analysis of the microplastic-associated chemical additives. In this study, a previously described density separation unit and protocol were assessed for the presence of polymer additives. The separation unit was constructed with clear polyvinylchloride (PVC) housing. A 1.5 g $cm^{-3}$ zinc chloride density solution was used so as to float heavier plastic types such as PVC. The recovery of spiked microplastics was determined for the protocol, as well as the associated chemical additives. The supernatant and remaining sediment were analyzed for the presence of polymer additives. Environmental sediment samples were collected from Ellerbe Creek, a local urban waterway. The samples were also separated by the tested protocol and analyzed for plastic-associated additives. Analysis included the use of a polymer additive and dye mass list in concert with the orbitrap lumos, as well as HPLC-ESI-MS/MS.

**WP064 Microplastics can impact the soil biophysical environment**

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Soils are essential components of terrestrial ecosystems that regulate a multitude of key environmental services such as biogeochemical cycling, biodiversity maintenance, and food security, among others. As a consequence, soils are directly impacted by multiple human activities to which they represent either the source or recipients of diverse common chemical and physical anthropogenic stressors. Microplastic contamination of soils is being increasingly documented, and its potential threats to terrestrial biodiversity and function have been recently conceptualized. Notwithstanding, data on effects of microplastics on fundamental properties potentially impacting soil biota are lacking. The present study explores the potential of microplastics to disturb vital relationships between soil and water, as well as its consequences for soil structure and microbial function. We exposed during 5 weeks a loamy sand soil to environmentally relevant nominal concentrations (up to 2 %) of four common microplastic types (polyacrylic fibers, polyester fibers, polyamide beads, and polyethylene high-density fragments). Then, we measured bulk density, water holding capacity, hydraulic conductivity, soil aggregation, and microbial activity. Diverse changes in the soil biophysical environment were observed. For instance, all microplastic types affected the bulk density soil. Both fibers significantly decreased microbial activity, while the polyester fibers increased the water holding capacity of soils. In addition, the soil structure, and the functional relationship between the microbial activity and water stable aggregates were also altered by microplastic exposure. The effects are underestimated if idiosyncrasies of particle type and concentrations are neglected, suggesting that purely qualitative environmental microplastic data might be of limited value for the assessment of effects on soil. If extended to other soils and plastic types, the processes unravelled here suggest that microplastics are relevant long-term anthropogenic stressors and drivers of global change in terrestrial ecosystems.

**WP065 Microplastics in the Mississippi River and Their Discharge to the Gulf of Mexico**

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Rivers are likely one of the greatest sources of plastic debris to the ocean, yet studies are just starting to quantify their contributions. The Mississippi River and its watershed encompass ~40% of U.S. land area and are home to >90 million people. Because of this large area and the plastic consumption potential of its population, the Mississippi River is likely one of the largest contributors of plastic debris to the global ocean. This research funded by the NOAA Marine Debris Program will quantify and characterize (size, shape, resin type) microplastics within the main stem and tributaries (Missouri, Illinois, and Ohio Rivers) of the Mississippi River near major cities such as St. Louis and New Orleans. Sample processing is ongoing, but preliminary estimates for the number of microplastics in the river and those potentially discharged to the Gulf of Mexico annually are in the quadrillions. Additional information that will be presented includes polymer type, spatial distribution and the influence of river volume on microplastic concentrations. The broader goal of this work is to create a baseline that can be used to guide fate and effects research as well as aid federal and local policymakers in creating and assessing mitigation strategies to improve water quality.

**WP066 Microplastics in the Mississippi River System**

*A. Scircle, University of Mississippi; J. Cizdziel, University of Mississippi / Chemistry*

Microplastics (MPs) concentrations on the inner shelf of the northern Gulf of Mexico (nGoM) are among the highest levels reported globally. Because their size range overlaps that of zooplankton, they are confused with prey and are accumulating in the food chain. The plastic particles are causing deleterious effects on aquatic organisms, particularly filter-feeders such as oysters. Moreover, plastics attract (sorb) certain contaminants, such as persistent organic pollutants and mercury, and thus their accumulation in biota may be an overlooked source of contaminants to ecosystems. This is a major concern to the state and region because seafood is a vital industry for Gulf Coast states, and because, on average, Gulf Coast residents consume more seafood than other U.S. residents. It is also a national problem because the majority of microplastics in the nGoM originate from the Mississippi River (MR), whose basin encompasses thirty one states. Yet, surprisingly little is known about the concentrations, types, sizes, and loadings of microplastics in the MR and its major tributaries. This lack of data is hindering our understanding of the magnitude and sources of the problem. This project aims to systematically quantify the concentrations and loads of MPs and characterize their shapes, size distribution, and chemical composition in the MR system—a source of drinking water to over 18 million people. We are collecting MPs using nets specifically designed for them and are using flow gauges to determine MP concentartions and loadings in the rivers. Wa are extracting and isolating the MPs using standard methods, including filtration, chemical treatment, and density separation. We are using fluorescence microscopy and µ-spectroscopic imaging to detect and identify the sizes, shapes, and composition of the MP contamination, including small MPs that are often overlooked. Here we will provide preliminary results for this ongoing work, determining the sizes, resin-type, and loads of MPs in the river system. This is important to assess possible relations between MPs levels and characteristics with sources and different watershed attributes.

**WP067 Microplastics loading estimation from urban areas to sewage treatment plants based from a monitoring campaign in Japan**

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One of a main land-based sources of micropalstics released to aquatic environment is considered to be human activities such as our daily life, industrial activities, agricultural activities and so on. These microplastics are released to wastewater and runoff water from sources and finally flow into sewage treatment plants (STWs). To control the concentration in surface water, it is important to estimate the load from urban areas to STWs and reveal the removal rates at STWs. In this study, a microplastics monitoring campaign is performing in order to measure the concentration in raw wastewater samples and effluent water samples at approximately 20 STPs in Japan. The concentration variations in raw wastewater samples during a day are investigated. Microplastics in raw wastewater and effluent water samples are analyzed by a new method using density separation, coagulation process and μFTIR with imaging system and multi-regression analysis. Each polymer type microplastics less than 100μm are qualified and quantified into each shape types such as fragments, spheres, fibers and so on. Based on these data, microplastics loading per capita to STWs can be estimated statistically. Removal rates are also estimated at Japanese STPs. These data will be used to simulate their concentrations in rivers and bays near large cities by simulation models. This presentation will show some results in this monitoring campaign.

**WP068 Microplastics: Are they an ecological risk?**

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Not since the early days of assessing the impact of nanomaterials, has there been such a proliferation of research on a group of materials, as seen with microplastics (MPs). Widespread reports of trillions of MPs discharging into freshwaters, being a vector of contaminant bioaccumulation, and their toxicity quickly led to widespread banning of microbeads (one component of MPs). MPs are often reported in the same context as macro-plastics whose risks to large mammals, sea turtles, birds and fish are well known. The majority of research identifying adverse effects of MPs have only used microbeads, with exposures several orders of magnitude higher than the worst sites in nature. As with any substance, there can be no risk if there is no exposure. Nevertheless, there is a need for MP research addressing: 1) Risks in depositional sediments near urban wastewater discharges, particularly in regards to fibers which are much more common than beads; 2) Risk of very small MPs (sub-MPs) below 300 microns (e.g., anti-fouling paint chips and fibers); 3) MP and sub-MP ingestion in the presence of natural prey; and 4) Development of standard methods for sampling and quantifying MPs.

**WP069 Quantification of Benzotriazole Ultraviolet Stabilizers and Organophosphate Flame Retardants in Plastic Debris and Industrial Microplastics**

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Benzotriazole Ultraviolet Stabilizers (UV-BTs) and Organophosphate Flame Retardants (OPFRs) are used as additives in plastic products, added to prevent discolouration due to UV radiation and to comply with flammability standards, respectively. Mimico Creek is a highly urbanized watershed in Southern Ontario, Canada, which drains into Lake Ontario. The prevalence of UV-BTs in particles suspended in the water of Mimico creek has been previously shown, with the concentrations being the highest ever reported in the literature for this type of sample. However, the origin of these compounds in the creek is not clear. There is a high incidence of informal disposal of plastic waste within the Mimico Creek watershed, and furthermore, plastics manufacturing in Canada is concentrated in Southern Ontario and microplastics have been found to be prevalent in sediments collected from Lake Ontario tributaries. These present a possibility that UV-BTs are being leached out of plastics and into the creek. Some OPFRs have also been detected in surface water in Ontario, and these were also investigated since both groups of contaminants are found in similar products. This work aimed to quantify the UV-BT and OPFR content of plastic debris collected from Mimico Creek, and of pre-production plastic pellets and to infer whether these plastics serve as a source or a sink for these contaminants in the creek. Preliminary results indicate that the concentrations of UV-BTs and OPFRs in the plastics are too low (in the ng/g range) to make them viable sources. Additionally, the composition observed for UV-BTs are mirrored in the plastics and the creek water, further indicating that they are being sorbed onto the plastics from the water. We also observe that different types of polymers show varying affinities for sorption of UV-BTs. The trends for the OPFRs were similar, but not completely identical to Ontario surface water OPFR concentrations.

**WP070 Sorption behavior of NSAIDs on microplastics in freshwater environments**

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High disposability, high durability and indiscriminate use have lead to the accumulation of plastics at uncontrolled rates in the environment. However, in the environment plastics are not the only source of water pollution; other contaminants can interact with the plastic debris. Studies have found that microplastics are able to adsorb and accumulate waterborne contaminants such as metals and hydrophobic organic compounds. Nontheless, there is a lack of data regarding the sorption of other important contaminants like pharmaceuticals and personal care products. Nonsteroidal anti-inflammatory drugs (NSAIDs) also known, as painkillers, are a group of pharmaceuticals widely and highly consumed in the market due to a low price and over the counter accessability. NSAIDs are frequently detected in the environment at μg/L concentrations. Furthermore, various studies have observed toxic effects of NSAIDs on various aquatic organisms at these same concentrations. Therefore, adsorption of NSAIDs on microplstics may result in long range transport and toxicity to aquatic organisms if ingested. Naproxen, ibuprofen and diclofenac have Log Kow values (3.18, 3.97 and 4.51) in the same range as persitant organic pollutants reported to have high partition coefficients with different microplastics. In the present study, we examine the sorption of three NSAIDs (ibuprofen, naproxen, diclofenac) with three types of microplastics (polystyrene (PS), ultra-high molecular weigth polyethylene (UHMWPE), and average molecular weight meduim density polyethylene (MDPE)) under freshwater conditions at different pHs. To investigate this process, isotherms will be determined using a Batch Equilibrium approach. Scanning Electron Microscopy (SEM) and Dynamic Light Scattering will be used to charcaterize the microplastics. It is expected that NSAIDs will be highly sorbed to UHMWPE, MDPE and PS microplastics, and sorption will be significantly correlated with Log Kow. Therefore, it is expected that distribution coefficients will be ordered in the following manner diclofenac (4.51) > ibuprofen (3.97) ≈ naproxen (3.18).

**WP071 The impact of carbon nanotubes (CNT) and ultraviolet light on the release of polymer additives from their microplastic matrices into the environment**

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Engineered nanomaterials and nanoparticles are increasingly used in consumer products due to their properties and ability to be placed into composites. Nanoparticles such as carbon nanotubes are placed into polymer matrices to improve tensile strength, UV inhibition, flame resistance, electrical and thermal conductivity and reduce gas permeability. Many studies have investigated the impact of release of these nanoparticles into the environment and their ecotoxicological effects. However, few studies have investigated how the inclusion of carbon nanotubes in polymer matrices can impact the release of polymer additives. Polymer additives can be released through mechanisms of pH-mediated hydrolysis, oxidation, UV degradation, mechanical abrasions, and thermal degradation. The release of these additives into the environment are of concern due to additives like Bisphenol A and nonylphenol which are known to act as endocrine disruptors. In this study, a 5 day leaching experiments of cryomilled epoxy containing varying concentrations of carbon nanotubes (0, 0.01, 0.05, 0.1%) was completed. The three polymer additives that were studied are Bisphenol A (BPA), Bisphenol A diglycerol ether (BADGE), and Nonylphenol. The epoxy was placed in water under conditions of no light, and a temperature of 65 °C. This experiment was performed with one set of epoxy exposed to 5 days of 500 Watt UV exposure, while a control was run in parallel where epoxy was not exposed to UV. Daily measurements of polymer additive concentrations were taken to investigate how the concentration of carbon nanotubes and UV exposure increase the release of these polymer additives into an aqueous environment.

## Improving Chemical Assessments Using Toxicokinetic Data

**WP072 In vitro-in vivo and cross-life stage extrapolation of uptake and biotransformation of benzo[a]pyrene in the fathead minnow**

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An ever-increasing number of chemicals are used in our society that are eventually released into the environment. Current regulations to assess toxicological risks associated with these chemicals rely on extensive live-animal testing. Because of the costs and ethical concerns with regard to animal testing, the 3R principle (i.e., reduction, replacement, refinement) demands that animal experiments should be substituted with alternative test methods whenever possible. Computational models have been proposed as powerful in silico alternatives to animal experiments. Among these, toxicokinetic (TK) models have received particular attention because they can be used to predict the time course of a toxicant's concentration at the target site. Thus, they are not only useful to inform bioaccumulation assessments, but also help interpreting and extrapolating toxicological effect data. Current TK models are shown to be highly accurate for neutral organic chemicals if species specific chemical biotransformation is accounted for. Potential inter-species and life stage differences in biotransformation kinetics, however, are currently not well-understood. To bridge this gap, the goal of this study was: (a) to characterize in vitro transformation kinetics of the rapidly biotransformed model chemical benzo[*a*]pyrene (BaP) during different life stages of the fathead minnow (*Pimephales promelas*) using the S9 stability assay, (b) to develop and apply TK models for early life stage and adult fathead minnows to extrapolate biotransformation from in vitro to in vivo, and (c) to validate model predictions using data from in vivo flow-through exposures to graded concentrations of water-borne BaP. In addition to the study of substrate depletion, we will also account for life stage-dependent relative abundances of different BaP metabolites through ultra high-performance liquid chromatography and high resolution accurate mass mass spectrometry (UPLC-HRAM MS). Because different BaP metabolites have been shown to be associated with different toxicological effects, our models may also help to understand life stage-dependent differences in toxicological effects. In a next step, we will also characterize inter-species differences in biotransformation. Based on data acquired to date, we conclude that toxicokinetic models in combination with in vitro assays are a powerful alternative to in vivo experiments and can also account for life stage-specific differences in biotransformation.

**WP073 Quantitative in vitro to in vivo extrapolation of biotransformation rates for bioaccumulation assessment: Focus on organic sunscreen agents in trout**

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Recent studies demonstrate the utility of in vitro to in vivo extrapolation (IVIVE) approaches for estimating the impact of biotransformation on chemical bioaccumulation in fish. However, rigorous evaluation of the IVIVE approach requires studies that generate well-matched in vitro and in vivo data from the same species. Here we present results from both in vitro and in vivo experiments that measured biotransformation rates of two organic sunscreen agents (ultraviolet filters; UVFs), ethylhexyl trimethoxycinnamate (EHMC) and octocrylene (OCT) (log $K_{OW}$ of 5.8 and 6.9, respectively) in rainbow trout. For the in vivo studies, trout were exposed to three dietary concentrations of each UVF to investigate the relationship between dietary exposure concentration and observed accumulation and elimination. EHMC and OCT were significantly metabolized, resulting in mean in vivo biotransformation rate constants ($k_{MET}$) of 0.35 ± 0.05 and 0.09 ± 0.01 $d^{-1}$, respectively. In vivo $k_{MET}$ values did not differ between dietary exposure concentrations, indicating that UVF concentrations in the fish were not high enough to saturate biotransformation enzymes. In vitro biotransformation rates were measured in hepatic and intestinal S9 fractions under demonstrated first-order conditions ($C_i << K_M$). These values were then extrapolated to the whole animal to estimate $k_{MET}$. The $k_{MET}$ values determined for EHMC and OCT using in vitro data (hepatic plus intestinal) exhibited good agreement with measured values. Importantly, however, these comparisons suggest that biotransformation of EHMC occurs largely in the liver, while intestinal biotransformation plays a major role in elimination of OCT. Finally, the fraction unbound ($f_U$) was measured in S9 fractions and blood plasma for EHMC, OCT, and several other hydrophobic test chemicals representing a range in $\log_{KOW}$ (4-8). Measured $f_U$ values were then compared to predictions generated using available binding algorithms. We conclude that future use of quantitative IVIVE methods for bioaccumulation assessment requires greater consideration of extrahepatic metabolism and improved algorithms for estimating unbound chemical fractions in vitro and in blood.

**WP074 Assessing biotransformation and bioconcentration factors of fragrance materials using in vitro approaches**

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Bioaccumulation potential measured as the bioconcentration factor (BCF) is one of the components for the PBT (persistent, bioaccumulative, toxic) criteria and risk assessment of chemicals by regulatory agencies in some regions of the world (e.g. REACH and ECHA). Biotransformation of chemicals is the largest source of uncertainty in bioaccumulation assessment. Currently, in vitro methodologies utilizing the rainbow trout metabolic assay not only are gaining interest, but are being used increasingly by several sectors as a crucial component in model-based estimates of BCFs and as part of a line of weight of evidence presented to regulators. The rainbow trout metabolic assay utilizing liver S9 fractions and cryopreserved hepatocytes to test chemical biotransformation has gone through a Ring Trial for an OECD validation process (OECD Project 3.13 coordinated by ILSI HESI) and the Test Guidelines and Guidance are being reviewed by the OECD assigned panel. In the present study four fragrance materials (Cyclabute, Melafleur, Trimofix and Verdox) with known measured BCF values obtained using OECD 305 method were tested for biotransformation utilizing both rainbow trout liver S9 fractions and cryopreserved hepatocytes. The results indicate that all four fragrance materials were metabolized in both biological systems at different rates, but in all cases the BCFs determined were comparable to the measured in vivo BCF values. The in vitro metabolic assay is a powerful tool that can be used to determine BCF of test chemicals and provide data to build the database information on fragrance materials for fish metabolism and modeling.

**WP075 Multi-compartmental toxicokinetic modeling of fipronil in tilapia: Accumulation, biotransformation and elimination**

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Bioaccumulation and biotransformation are the critical processes modifying the toxicity of easily metabolizable chemicals. In the present study, tissue-specific accumulation, biotransformation and elimination of a pesticide fipronil in tilapia (*Oreochromis niloticus*) were quantified by combining in vivo measurements and a newly developed multi-compartmental pharmacokinetic model. Waterborne fipronil was taken up via gills and metabolized rapidly and solely to fipronil sulfone, which had three-fold higher whole body concentration and 30-fold longer half-life in tilapia than the parent compound. The highest concentrations of fipronil and fipronil sulfone were detected in the liver, followed by intestine, gill, bile, carcass and blood. Significant decrease of fipronil residues in the liver and intestine during exposure period strongly suggested the metabolism in these two organs. Intestinal transformation of fipronil was as important as hepatic transformation in the waterborne exposure. Significant transport of fipronil and fipronil sulfone in the liver-bile-intestine system implied that hepatobiliary excretion and enterohepatic re-absorption played important roles in fipronil metabolism and system circulation of the parent compound and the metabolite. The multi-compartmental model quantitatively demonstrated the highly dynamic inter-compartmental transport and rapid branchial clearance of fipronil in fish. Modeling results also suggested that uptake and biotransformation were the stronger driving forces for the inter-compartmental transport of xenobiotics in fish than the inherent partitioning capacity.

**WP076 Parameter Optimization and Statistical Analysis of PBTK Models for Fluoranthene Exposure in Rainbow Trout**

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Physiologically based toxicokinetic (PBTK) modeling in fish offers great promise in environmental risk assessment, potentially speeding up dose-response studies while minimizing animal testing. Some limitations exist in the PBTK field, such as difficulty of model development and a lack of application specific software tools to help modelers. These limitations have slowed the progress of PBTK modeling for risk assessment. In a closely related field, physiologically based pharmacokinetic (PBPK) modeling has been used for drug development. Several recent publications have discussed the requirements for PBPK modeling in regulatory submission which are analogous to the needs of PBPK modeling. The requirements include standards for distribution and documentation of models, statistical methods for optimization and validation of models, databases for physiological parameters, and better software tools. Our previous research addressed some of these requirements by presenting Petri Nets as an open source, unified method for simulating, documenting, and distributing PBTK models in fish. The current research expands on this by offering an open source tool for optimizing PBTK parameters, performing sensitivity and variability analyses, and conducting statistical evaluations of the PBTK models. The software is demonstrated by comparing different PBTK models of fluoranthene exposure in rainbow trout. The models differ in the metabolizing compartments, where specific assumptions regarding phase I vs phase II metabolizing enzymes are tested. Various algorithms are compared to optimize parameters for each model. Statistical analysis results are used to determine the best fit among the models.

**WP077 General Unified Threshold Model of Survival (GUTS) Model of the Effects of Perfluorooctanic Acid on Loggerhead (*Caretta caretta*) Sea Turtle Cells**

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Loggerhead (*Caretta caretta*) sea turtles are a threatened species, and yet little toxicological research has been conducted on them. This study entails toxicity testing of Perfluorooctanic Acid (PFOA) on loggerhead sea turtle cells, followed by the production of a mathematical model that predicts survival based upon the empirical data. PFOA is a common component in non-stick coating on pans, a surface repellent in carpet, paper, and cardboard, and an ingredient in fire-fighting foam. This toxicant is known for its cytotoxicity, mutagenicity, immunotoxicity, adverse developmental effects, and negative impacts on renal and hepatic systems in multiple animal models, including reptiles. Cytotoxicity of PFOA on loggerhead skin was examined using the MTT viability assay to assess primary skin cell cultures exposed to environmentally relevant concentrations for 24, 48, 72, and 96 hours. This toxicological data was then used in the General Unified Threshold Model of Survival (GUTS) to provide a framework for the prediction of loggerhead sea turtle cell survivability overtime. GUTS delivered a guideline for parameterizing sound toxicokinetic toxicodynamic (TKTD) models that effectively looked at the sensitivity of loggerhead cells to PFOA. There are two survivability models that resulted from the use of GUTS – individual tolerance (IT) and stochastic death (SD). IT forecasted survivability based on the individual sensitivity of each cell; all cells have individual tolerance levels for the contaminant. The SD approach predicted survivability based on the idea that all cells share a common adverse effect threshold, and similarly have decreased likelihood of survivability at the same rate. The SD and IT approaches provided valuable and novel insight into the survivability risks PFOA poses to loggerhead sea turtles. Both SD and IT models were analyzed for effectiveness using confidence intervals and profile likelihoods. Each model served as a tool for conducting standard ecological risk assessments through the establishment of a fifty percent lethal concentration ($LC_{50}$) prediction at various exposure time points. These models provided the first GUTS models for this marine reptile and the first application of GUTS to cell populations rather than individual animals, pioneering the advent of TKTD models for endangered species that cannot undergo normal toxicity testing.

**WP078 Updated Models for Predicting Biotransformation Impacts on Chemical Bioconcentration in Rainbow Trout**

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Biotransformation may substantially reduce chemical accumulation in fish, and is a recognized source of uncertainty in many bioaccumulation assessments. Here we describe and compare two one-compartment toxicokinetic models that can be used to predict the effect of hepatic biotransformation on bioconcentration factors (BCFs) in rainbow trout (*Oncorhynchus mykiss*). Both models employ established methods to extrapolate in vitro intrinsic clearance rates measured using liver S9 fractions or isolated hepatocytes to the whole animal; however, one model relies of QSARs to predict rates of chemical uptake and elimination while the other describes these processes in physiological terms. Compared to the QSAR-based model, the physiological model more explicitly addresses how differences in fish size and water temperature affect rate constants for chemical uptake and elimination (e.g., branchial uptake) in the fish. In addition, the physiological model provides a more direct link to previously published estimates of whole-body biotransformation rate constants ($k_B$), derived from in vivo BCF and dietary bioaccumulation tests in various fish species. BCFs predicted by the physiological model in the absence of biotransformation are somewhat higher than those generated by the QSAR-based model, due to differences in predicted chemical partitioning. However, the physiological model predicts lower BCFs for compounds that undergo biotransformation at all but very low levels of biotransformation activity. This difference in predicted BCFs varies with log $K_{OW}$ and the biotransformation rate, and in some instances exceeds 1.5-fold ($BCF_{QSAR}/BCF_{Phys}$). The utility of the physiological model was demonstrated by calculating $k_B$s for a suite of polycyclic aromatic hydrocarbons using measured in vitro biotransformation rate data. The resulting $k_B$s exhibit remarkably good agreement with in vivo and in silico (QSAR) estimates. Finally, uncertainty in model selection for BCF calculation was put into context by highlighting variability and uncertainty in measured in vivo BCFs.

**WP079 Developing and testing models to integrate toxicokinetic data for assessing bioaccumulation in mammals**

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Historically, the globally accepted model for bioaccumulation (B) assessment has been fish, and B screening and assessment has been driven by the octanol-water partition coefficient ($K_{OW}$) and data and assessment endpoints for aquatic species; however, nearly two decades of evidence has highlighted the need to examine B in air-breathing organisms as well. Emerging regulatory guidance, e.g., REACH Chapter R11: PBT / vPvB Assessment, includes B screening threshold criteria for air-breathing organisms linked to $K_{OW}$ and the octanol-air partition coefficient ($K_{OA}$). It is also acknowledged that considerations of the dietary absorption efficiency of the chemical and biotransformation rates are also necessary for B assessment. Tools, models and data streams are emerging to aid in B assessment for mammals such as in silico models to predict chemical partitioning and biotransformation half-lives and in vitro and in vivo data that can be used to parameterize toxicokinetic (TK) models such as in vitro biotransformation rates estimated from hepatocytes. While certain tools for simulating B in mammals and other air-breathing organisms exist, it is desirable to further develop and refine tools and data streams more explicitly and to evaluate (test) these frameworks. This project expands the development and evaluation data and models for B and exposure and risk assessment for mammals. A particular focus of this project is to address data gaps and uncertainty in biotransformation half-lives. The initial objective of the project is to assimilate critically evaluated data from various sources of in vitro and in vivo TK data (rodents and humans), as well as field B data in mammals (e.g., biomagnification factors). This poster will describe initial efforts to assimilate these data, as well as highlight future project goals and tasks (i.e., exploring new Quantitative Structure-Activity Relationships (QSARs) to predict biotransformation rates and other key TK data).

**WP080 Literature-Derived Bioaccumulation Models for Polychlorinated Biphenyls in Plants and Small Mammals**

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We reviewed literature on the bioaccumulation of polychlorinated biphenyls (PCBs) in plants and small mammals, to support the wildlife exposure characterization for an ecological risk assessment. Suitable field studies for this analysis reported soil and whole-body (or similar) tissue PCB data from the same site, and all studies included were reviewed for appropriate study design, documentation, and data quality. For plants, extensive data are available from phytoremediation research and other studies; we selected five representative field studies that provided data for multiple non-agricultural plant species. For small mammals, a comprehensive review of peer-reviewed and gray literature was necessary to obtain an adequate data set, and seven field studies were identified. Bioaccumulation regressions were selected over point-estimate bioaccumulation factors for application in the food web model, if a statistically significant linear relationship ($p< 0.05$) existed between soil and tissue PCB concentrations, because such regressions provide a more robust estimate of bioaccumulation across a range of soil concentrations. Due to limited availability of lipid and organic carbon data, analyses were performed without normalizing for these factors. Additionally, lipid and organic carbon normalized data were analyzed for a subset of the small mammal data, representing sites contaminated with less-chlorinated PCB mixtures. For plants, bioaccumulation of PCBs in wetland and upland species did not differ. Therefore, a single statistically significant regression equation based on all available data for wetland and terrestrial plants was used in the food web model. For small mammals, bioaccumulation of PCBs was found to be significantly higher in shrews than in other small mammals (i.e., mice and meadow voles), consistent with the higher trophic position and soil ingestion rates of shrews compared to other small mammals. Therefore, bioaccumulation was estimated separately for shrews and other small mammals, and the regressions were both statistically significant and used in the food web model. The resulting bioaccumulation models were sufficiently robust to allow site-specific sampling to focus on other food web components with higher bioaccumulation potential, without the need to sample and analyze site-specific PCB concentrations in plants and small mammals.

**WP081 Multi-Species Approach in Physiologically Based Toxicokinetic (PBTK) Modelling in Support of Ecological Risk Assessment**

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The production and release of chemicals by our society has been described as one of the greatest threats to the sustainability of human activities on this planet. Legislations of varying rigor have been implemented globally and seek to minimize impacts of chemicals on the environment through ecological risk assessments (ERA). The foundation of ERA are standardized toxicity data which are generated in laboratory experiments with model species. However, these species are not necessarily representative of native species of concern in an ecosystem. Furthermore, a wealth of data from non-model species is available in the scientific literature but cannot be utilized in ERA because the data were not derived in compliance with test guidelines. Therefore, approaches are needed that enable inclusion of these data, such as models, that enable transposing these datasets into a format that is useful in ERA. One class of models that facilitate extrapolation between levels of biological organization, exposure conditions, and among species are physiologically based toxicokinetic (PBTK) models. In our previous research we successfully re-parameterized a single-species PBTK model for multiple species and integrated this model into a multispecies modeling framework. In contrast to this "top-down" approach, here we present a "bottom-up" multispecies PBTK modeling framework which will be based on available data from the 222 freshwater fishes found in Canada. This approach – unlike all previous models – does not require full sets of model parameters to define individual species fully but will rather make use of a database of all available data to describe the statistical distributions of model parameters. These distributions are then used to feed into random number generators in stochastic Monte Carlo simulations to make probabilistic cross-species toxicokinetic predictions. In addition to enabling cross-species extrapolations in ERA, our novel stochastic multispecies PBTK model provides a framework that can help address various environmentally relevant questions by providing predictions for specific taxonomic, ecological, or geographic groups of fishes. As such, our new model will potentially enable more environmentally relevant predictions using already existing data, and could ultimately lead toward more sustainable use of existing data.

**WP082 The Bioaccumulation Assessment Tool (BAT): A quantitative weight of evidence approach for bioaccumulation assessment**

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Chemicals are assessed for bioaccumulation (B) potential in regulatory programs using various methods, metrics and criteria. The Bioaccumulation Assessment Tool (BAT) was developed to standardize the collection, evaluation and the integration of various lines of evidence (LOE) with available toxicokinetic (TK) information and related B classification criteria to aid decision-making. The BAT provides a transparent and consistent framework for the evaluation of neutral and ionizable organic chemicals in aquatic and terrestrial organisms. It uses a quantitative weight of evidence (QWOE) approach which includes assessments for the relevance, reliability (confidence) and outcome of each B-metric source. Each substantive LOE (e.g., bioconcentration factor (BCF), biomagnification factor (BMF), trophic magnification factor (TMF), biotransformation rate, etc.) is subject to a standardized data quality evaluation resulting in a data reliability score by which the outcome of the LOE can be weighted. The Data Evaluation Templates (DETs) have been derived from standard test protocols (OECD) and expert judgment when standard protocols are not yet developed. Physical-chemical properties can be used or the user may enter biologically-relevant partition coefficients in place of default assumptions that assume octanol as surrogate for biological components (i.e., lipid). Estimates for biotransformation rates can be included from in vitro assays (i.e., S9, hepatocyte, microsomal), from in silico (QSAR) and in vivo study predictions. Empirical, in vivo study data including lab BCFs and BMFs and field data as well as in silico data (e.g., BCF-QSARs) can be assessed for reliability and included in the QWOE. We provide an overview of the BAT and demonstrate its application with two case studies. The first example is a typical "data poor" scenario in which only chemical structure information is available. From chemical structure relevant physical-chemical property and biotransformation rate data are obtained from QSARs and entered into the BAT. The second case study is for a relatively "data rich" chemical for which various LOE exist (e.g., 3 lab BCFs, various BCF-QSARs, biotransformation rate QSARs, in vitro biotransformation rates).

## Emerging Flame Retardants: State-of-the-Art Knowledge and Addressing Research Gaps

**WP083 Emerging flame retardants: A review of current policy approaches and critical research needs to inform evidence-based policy**

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The phase out of PBDEs and HBCDD resulted in a large number of halogenated and non-halogenated chemicals being used as replacement flame retardants in furniture, children's products, electronics and building materials. Studies find almost 50 unique replacement flame retardants in U.S. indoor dust samples alone. Chemical policies have generally managed flame retardants through a paradigm of allowing use while conducting an in-depth, quantitative risk assessment of specific chemicals to inform risk management. Risk assessments include studies of human exposures and effects, and if the chemical is found to be harmful, health damages may already have occurred prior to regulatory decisions. Further, risk management has usually focused on ceasing manufacture and import while not addressing existing products containing flame retardants. This is especially problematic for persistent and bioaccumulative chemicals where products in situ, disposal and recycling will result in the chemicals' presence and exposures for decades after manufacture is banned. However, there is increasing recognition that this paradigm is insufficient to address current chemicals and their exposures before harm or keep pace with the proliferation of new chemicals. This presentation will review new policy approaches being taken by businesses, cities, states, countries and international bodies to attempt to address existing and emerging flame retardants such as: disclosure of information on flame retardants in products; considering classes of flame retardants instead of single chemicals; regulating recycling/ disposal of flame-retarded products; and alternatives assessments for flame retardants. For example, the 2016 Lautenberg amendments to the U.S. Toxic Substances Control Act mandate that exposures to persistent, bioaccumulative and toxic (PBT) chemicals be eliminated to the extent practicable—meaning that for the first time, the Environmental Protection Agency is required to assess existing products containing PBT flame retardants. The Consumer Product Safety Commission is currently evaluating scientific approaches for considering all halogenated flame retardants as a class for regulation. The types of scientific information needed for new policy efforts will be compared and contrasted with what is needed for risk assessment, with recommendations for making research on emerging flame retardants relevant and translatable to inform policy.

**WP084 Greener Fire Safety Solutions for Interior Automotive & Building Insulation**

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For several decades, there have been numerous peer reviewed scientific studies documenting human health and environment concerns of certain organohalogen flame retardants. TDCPP and TCPP are organohalogen flame retardants used in large volume within North America (re. automotive, Building & Construction). The U.S. Consumer Product Safety Commission has recently expressed concerns about the use of organohalogen FRs in certain consumer goods. This presentation will review two large volume FR applications where organohalogen flame retardants are used (interior automotive, building insulation). The audience will be informed that Greener FR solutions are already commercially available which meet existing fire safety requirements.

**WP085 First Steps Towards Characterization of Polyhalogenated Alkane (PXA) Flame Retardants**

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Brominated/chlorinated alkenes have been used for over 20 years as flame retardants, but are not currently registered in Europe under REACH. As polyhalogenated alkanes (PXAs), they are expected to resemble chlorinated paraffins in toxicity and environmental behavior. The objective of this work is to characterize a commercial brominated/ chlorinated alkene product as an initial step towards understanding the environmental occurrence and persistence of PXAs. A sample of Doverguard 8207, purportedly containing $C_{12-30}$ brominated/chlorinated alkenes (CAS# 68527-01-5) was run by four different high resolution techniques: GCxGC-EI(+)-HRMS, GC-APCI(-)-HRMS, LC-APCI(-)-Orbitrap MS, and FTICR-MS in negative mode. Medium (MCCP) and long chain (LCCP) chlorinated paraffins were also analysed for comparison. Analysis by GCxGC-EI(+)-HRMS results indicated that 68527-01-5 consisted mainly of chains lengths of 18 or more carbons, substituted with several chlorine and bromine atoms. GC-APCI(-)-HRMS yielded five prominent PXA ions with proposed elemental compositions of $C_{18}H_{31}Br_2Cl_4$-, $C_{18}H_{31}BrCl_5$-, $C_{18}H_{30}BrCl_6$-, $C_{18}H_{30}Br_2Cl_5$-, and $C_{18}H_{29}Br_2Cl_6$-. Although molecular ions were not observed for several of these PXAs, their identification was confirmed through FTICR-MS analysis. The addition of DCM between the source and column resulted in exclusive formation of [M+Cl]- adducts for the CP standards, in agreement with previous studies. The most prominent PXA ions identified by LC-APCI(-)-Orbitrap MS, in order of decreasing abundances, were $C_{18}H_{32}Br_2Cl_5$-, $C_{18}H_{31}$Br2$Cl_6$-, $C_{18}H_{30}Br_2Cl_7$-, $C_{18}H_{31}Br_3Cl_5$-, and $C_{18}H_{33}Br_2Cl_4$-. LC-Cl-enhanced APCI (-)-Orbitrap appears to form [M+Cl]- ions, and corroborates the formation of either [M-H]- or [M-X]- ions in GC-APCI(-)-HRMS, based on the prominent PXA ions observed in both methods. The data obtained so far confirm industry information that this substance has a Cl:Br ratio of ~2.6:1 but do not entirely support the data description of the product as $C_{12}$-$C_{30}$ bromo/chloro-alkene. Assuming that bromo-chloro-$C_{18}$ alkanes are the major constituents, 68527-01-5 contains very hydrophobic molecules. Log Kows for the main $C_{18}$ alkanes ions in 68527-01-5 were estimated by correlations with LC retention time of a suite of 21 hydrophobic organics. Estimated log Kow for major Br/Cl-PXA components with C18 chains ranged from 6.4 to 6.9. This was lower than estimated using KOWWIN for representative Br/Cl-C18 PXAs which ranged from 10-11.

**WP086 Measurement of physicochemical properties of EH-TBB, BEH-TEBP, and TPHP, and differences in their properties between pure substance and mixture**

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Recently, environmental occurrences of non-PBDE (polybrominated diphenyl ethers ) brominated flame retardants (BFRs) such as “novel BFRs,” “emerging BFRs,” etc., have been reported by various research groups. In order to understand the environmental fate and mechanism of contamination of these chemicals, knowledge of their physicochemical properties, such as vapor pressure ($p_i$), water solubility ($S_w$), and 1-octanol–water partition coefficient ($K_{ow}$) is necessary. Therefore, we have measured these properties for several non-PBDE BFRs. In this study, we newly measured the properties of 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB) and bis(2-ethylhexyl)-3,4,5,6-tetrabromophthalate (BEH-TEBP). The following is a brief summary of the methods used to measure $S_w$, $K_{ow}$, and $p_i$ values. The $S_w$ and $K_{ow}$ at 298 K were measured with a direct coupled column linked chromatographic technique and the slow stirrer method, respectively. We used the generator column method for the $p_i$ measurement at elevating temperatures. The $p_i$ value at 298 K ($p_i^o$) was determined by fitting the vapor pressure data with the Clausius−Clapeyron equation. Compared with some PBDEs and non-PBDE BFRs measured in our previous works, $p_i^o$ values of EH-TBB ($6.20\cdot10^{-6}$ Pa) and BEH-TEBP ($1.08\cdot10^{-8}$ Pa) were similar to those of BDE-99 (PBDEs with 5 bromines, $3.67\cdot10^{-6}$ Pa ) and BDE-153 with 6 bromines ($1.28\cdot10^{-8}$ Pa), respectively. In terms of log $K_{ow}$ and $S_w$, however, EH-TBB (7.02 and $6.15\cdot10^{-4}$ mg/L) and BEH-TEBP (7.65 and $1.19\cdot10^{-6}$ mg/L) were lower than BDE-99 (7.35 and $3.47\cdot10^{-3}$ mg/L) and BDE-153 (8.05 and $5.04\cdot10^{-5}$ mg/L) , respectively. EH-TBB and BEH-TEBP are main BFR components in a commercial product Firemaster 550. This product also contains another organophosphorous flame retardants such as triphenylphosphate (TPHP). Actually, a liquid mixture of those compounds is used. In this study, we investigated if there are any differences between pure state and mixture states in terms of physicochemical properties. We expected the differences could not be predicted by the ideal solution assumption, since the activity coefficient values of the three flame retardants in their mixtures estimated by COSMOtherm were higher than unity. Therefore, we measured the properties for pure TPHP, and then tried to measure $p_i$ and $S_w$ of the three components in a mixture state. At our presentation, we show the difference among the pure and mixed-state properties.

**WP087 Investigation of flame retardants incorporated into plastic enclosures of liquid crystal display monitors for personal computers on Japanese market**

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The restriction against polybrominated diphenyl ether (PBDE) flame retardants (FRs) according to the RoHS Directive in the EU was introduced the increasing application of alternative halogenated FRs (AHFRs) and organophosphorus FRs (PFRs) in polymeric materials of electronic products all over the world. However, emerging concerns are raised about environmental contamination and human health risks posed by AHFRs and PFRs. Information on the types and concentrations of FRs in polymeric materials of electronic products currently on market is crucial for the assessment and prediction of environmental contamination and human health risks posed by FRs derived from the use and processing of electronic products. Nevertheless, no definitive information exists for the presence of FRs in plastic enclosures of liquid crystal display monitors for personal computers. We investigated the presence of PBDEs, AHFRs, and PFRs in the plastic enclosure samples collected from 45 liquid crystal display monitors for personal computers by GC-EI-QMS and LC-ESI-MSMS measurements. These monitors were manufactured by 15 display technology companies in China, Japan, South Korea, and the United States through 2001 to 2013 and, consequently, they were put on Japanese market in 2015. Of the 45 monitors, all the monitors measured in this study have fulfilled the RoHS threshold for PBDE concentrations (0.1%). Two monitors manufactured before the RoHS Directive were determined to contain tetrabromobisphenol-A with the same concentrations of 14%, whereas two monitors manufactured after the RoHS Directive were determined to contain two AHFR mixture including decabromodiphenyl ethane and 2,4,6-tris(2,4,6-tribromophenoxy)-1,3,5-triazine with the total concentration range from 13 to 14%. The 21 monitors manufactured over the course of the period between 2001 and 2011 were determined to contain three oligomeric PFRs, such as bisphenol A bis(diphenyl phosphate), resorcinol bis(diphenyl phosphate), and resorcinol bis(dixylyl phosphate), with the concentration range from 4.4 to 9.2%. The remaining 20 samples were expected to contain unknown halogen-free FRs on the basis of the results from element screening using a handheld X-ray fluorescence analyzer.

**WP088 Nationwide Distribution of 19 Organophosphate Flame Retardant (OPFRs) in Indoor Dust, Outdoor Dust and Soil in China**

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Organophosphate flame retardants (OPFRs) have received attention in recent years due to their widespread usage (e.g., in plastics, electronic products), increasing production (>680,000 tons/year), occurrence in environmental matrices and adverse impact on human health. A few studies in China showed elevated contamination by OPFRs in certain localized regions. However, the studies on nationwide distribution of OPFRs are limited. In the present study, 19 OPFRs were measured in 44 indoor dust (ID), 97 outdoor dust (OD) and 100 soil (S) samples collected from different regions of China. ∑19OPFRs level in indoor dust (2380 ng/g; median) was much higher than outdoor dust (446 ng/g) and soil (36.4 ng/g) samples. OPFR concentrations in outdoor matrices showed a positive correlation with the population density of various regions in China. Northeastern China with the traditional industrial base, and the Eastern and Southcentral China with high population density and economic development had significantly higher levels of OPFRs than the other areas. Samples from Guangdong province, which is the world center for electronic waste recycling, contained extremely high OPFR level (ID:5700 ng/g), indicating that e-waste is still an important source of OPFRs. Tris(2-chloroisopropyl) phosphate (TCIPP) had the highest detected frequency (DF; >95%) and concentration (ID:690, OD:85.8, S:14.5 ng/g). Cresyl diphenyl phosphate (CDPP) and Isodecyl Diphenyl Phosphate (IDDP) were also detected (DF>80%) at considerable levels (ID:3.85-10.6, OD:2.04-5.48, S:0.39-0.14 ng/g) revealing their increasing usage in China. Aryl-OPFRs showed significant correlations ($r$=0.326-0.855, $p$< 0.05) with each other in all three matrices. There was a significant but weak correlation between OPFRs concentration in paired indoor-outdoor dust ($r$=0.475, $p$< 0.01) and outdoor dust-soil ($r$=0.437, $p$< 0.01) samples. Adults intake (EDI) through dust ingestion was estimated at 0.55 ng/kg bw/d ∑19OPFR while children intake at 6.84 ng/kg bw/d. EDI values from outdoor environment were less than 5% of that from indoor and the dust ingestion was the major pathway (>98%) of OPFRs exposure.

**WP089 Occurrence of OPFRs/plasticizers in indoor air and the associated human exposures**

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The occurrence and profiles of 15 organophosphate flame retardants/plasticizers (3 chlorinated [Cl]-OPFRs, 3 aryl compounds, 9 non-Cl alkyl and other compounds) were investigated in indoor air and dust from various types of microenvironments (total n=64) in and around the Albany area of New York State. Indoor dust from the floor was collected from 3 houses and 3 fire stations to investigate partitioning characteristics of OPFRs/plasticizers between vapor phase and particulate phase of air and in dust. The total concentrations of organophosphate esters (OPEs) in bulk air were found at several tens to hundreds of ng/m3. Among the eight categories of microenvironments, samples collected from automobile parts shops contained the highest concentrations of OPEs in indoor bulk air (mean: 258 ng/m3), followed by electronics shops, nail salons and interior design shops. Daily intake of OPEs via the inhalation, dermal contact, and ingestion of indoor air/dust will be presented.

**WP090 An assessment of the exposure of the Irish population to selected brominated flame retardants via indoor air and dust**

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This study assesses for the first time, exposure of the Irish population to polybrominated diphenyl ethers (PBDEs), hexabromocycododecane (HBCDD), and decabromodiphenyl ethane (DBDPE) in common indoor microenvironments. To do so, passive air sampler (PAS) and floor dust samples were collected from Irish homes, cars, offices and primary school classrooms (n=30 per microenvironment) between August 2016 and January 2017. PAS consisting of a sorbent (XAD-3) impregnated polyurethane foam disk (PUF) were deployed for 60 days, placed on elevated surfaces in homes, offices, and schools, and the floor behind the passenger or driver's seat in cars. Dust samples were collected by vacuuming floors following methods previously described. Concentrations in both air and dust of PBDEs and HBCDD in Ireland were of similar magnitude to those reported previously for the UK. In contrast, concentrations of DBDPE in air (median: 100 pg/m$^3$, range: 0.72-7000 pg/m$^3$) and dust (6900 ng/g; range: < 6.0 – 540000 ng/g) are to our knowledge the highest reported globally to date. These data suggest that DBDPE is being used substantially in Ireland.

**WP091 Analysis of Polybrominated Diphenyl Ether, Novel Brominated Flame Retardants and Organophosphate Flame Retardants in Dust from Canadian Fire Stations**

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The long term and widespread use of flame retardants (FRs) such as polybrominated diphenyl ethers (PBDEs) and their replacements such as novel (non-PBDE) brominated flame retardants (NBFRs) and organophosphate flame retardants (OPFRs), as well as their potential for environmental and biological adverse effects, have been exhaustively documented. House dust is one matrix that contains very high levels of many persistent organic pollutants (POPs), including some of these FRs discussed above, and one source of house dust of particular interest is dust from the living quarters of fire stations. By nature of their jobs, firefighters are exposed to a wide variety of contaminants at fire events, which they can carry back to the station on their turnout gear. In addition, their living quarters contain the same products that civilians have in their homes (e.g., upholstered foam furniture, computers and other electronics), which also potentially contribute to high levels of FRs in dust. Thus, firefighters are exposed to these high levels of FRs, as biomonitoring studies have demonstrated. In coordination with the International Association of Fire Fighters (IAFF), vacuum cleaner bags from vacuums used to clean 24 fire station living quarters from four different Canadian provinces (British Columbia, Alberta, Manitoba, Ontario) were sent to our laboratory (2018), along with questionnaires regarding station characteristics, operations and practices. Contents were sieved and the fine fraction collected and used for analysis for: (1) PBDEs and NBFRs by high resolution GCMS; and (2) OPFRs by gas chromatography / tandem mass spectrometry (GC-MS/MS). Results from the Canadian samples are compared and contrasted with those obtained from our previous studies, including: (1) residential house dust collected from 204 Northern California homes (2001 – 2007, 2010); (2) dust from 20 Southern California fire stations (2010 – 2011); and (3) fire station dust from 25 fire stations (2015) from five different US states (California, Minnesota, New Hampshire, New York, and Texas). The views expressed are those of the authors and do not necessarily reflect the views of the State of California.

**WP092 Modelling the Fate of Organophosphate Esters in Toronto Stormwater**

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Semi-volatile organic compounds (SVOCs) are a class of chemicals released in large quantities in urban areas from a variety of diffuse sources. The traditional focus of SVOC regulation has been for compounds which are persistent, bioaccumulative and have the potential for long range transport (PBT), which include polychlorinated biphenyls (PCBs), organochlorine pesticides and polybrominated diphenyl ethers (PBDEs). These compounds are generally hydrophobic and are therefore not typically found at high concentrations in surface waters. However, this is not the case with other SVOCs that are now receiving greater attention, some of which being used as replacements for the more traditional SVOCs as their usage is restricted through regulation. Reemtsma et al., (2016) have referred to these compounds as persistent mobile organic compounds (PMOCs) and have proposed mobility (M) as the new source of concern to replace "B" (bioaccumulation) in the persistent-bioaccumulative-toxic paradigm. Organophosphate esters (OPEs) are an example of SVOCs that could be considered as PMOCs. OPEs can be categorized into chlorinated compounds (Cl-OPEs), which are largely used as flame retardants, and non-chlorinated OPEs (non-Cl-OPEs) that are used as flame retardants and plasticizers. We investigated the transport and fate of OPEs through Toronto, Canada by modifying the Multimedia Urban Model of Diamond and Co-workers (2001) to better represent the transport and fate of PMOCs. Currently, we are developing a general model of contaminant fate in BCs, called the "Bioretention Blues" model, in order to investigate the efficacy of BCs for reducing loadings of PMOCs to surface water ecosystems using OPEs in Toronto as a case study. Our results indicated that stormwater is an important pathway by which OPEs enter surface water ecosystems, accounting for between 88-96% of loadings to nearshore Lake Ontario delivered by Toronto streams. Our results show that soil is effective in preventing >80% of loadings of non-Cl-OPEs through transformation, but was much less effective for Cl-OPEs, degrading < 20% of inputs to the soil while the rest entered either ground or surface water. These results show that BCs may be effective in mitigating the loadings of non-Cl-OPEs, but may be less effective for Cl-OPEs. Improvements to BC design, such as the use of organic capturing amendments, may be necessary to mitigate the loadings of Cl-OPEs and other PMOCs.

**WP093 Presence and distribution of 25 emerging flame retardants from five wastewater treatment plants in Canada**

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In this study, we report on the occurrence and phase distribution of a diverse suite of emerging non-polybrominated diphenyl ether (non-PBDE) flame retardants (FRs) in the liquid and solid waste streams of five wastewater treatment plants (WWTP) in Canada. The flame retardants studied included 6 dechlorane and related FRs, Di(2-ethylhexyl) tetrabromophthalate (BEHTBP), 2-Ethylhexyl 2,3,4,5-tetrabromobenzoate (EHTBB), 1,2-Dibromo-4-(1,2-dibromoethyl)cyclohexane (TBECH), 4 tribromophenyl ether and related FRs, and 12 monoaromatic FRs.. Influent, effluent and biosolid samples were collected from 5 WWTP in Canada. The samples were analyzed using liquid-liquid or liquid-solid extraction with liquid-liquid and gel permeation cleanups (GPC) and GC-(ECNI)-MS instrumental method. Distribution of the analytes dissolved in the aqueous phase and adsorbed to the solid phase were measured and their partitioning will be discussed. The measured values will also be compared to measured concentrations of organophosphate and PBDE flame retardants from the same treatment plants. This data contributes to our understanding of the occurrence, fate, behaviour and relative environmental significance of some of the important emerging flame retardants.

**WP094 Development and testing of a novel method to measure tetradecabromo-1,4-diphenoxybenzene in biosolid sludge from Canadian waste water treatment plants**

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Tetradecabromo-1,4-diphenoxybenzene (tetradeca) is a highly brominated flame retardant which has been shown to degrade photolytically to produce lower brominated polybrominated-diphenoxybenzenes, compounds which show similar structural components of those reported as contaminants in the eggs of Great Lakes herring gull (*Larsus argentatus*). Currently there are no reports of tetradeca or its potential products in environmental samples including sediment in the Great Lakes area. Given the large size and hydrophobicity of tetradeca as well as its use in commercial flame retardant mixtures (SAYTEX 120), we hypothesize that biosolids from Canadian waste-water treatment plants (WWTPs) may be a sink for these compounds. The present study investigated tetradeca and some of its debrominated photolytic by-products in biosolid samples gathered from WWTPs that have previously shown to contain flame retardants. The biosolid samples were collected in 2014 to 2017 from 20 WWTPs serving urban centres across Canada. The biosolid samples were also from WWTPS differing in treatment type, including digestion (aerobic or mesophilic anaerobic), dewatering, alkaline stabilization and/or pelletization. A novel analytical method for measuring tetradeca on low-resolution mass spectrometry (MS) was adapted from a published high-resolution MS method while extractions utilized a Soxhlet extractor where previously accelerated solvent extraction was used. The newly developed method is more accessible to a wider range of laboratories, allowing further studies on tetradeca to be performed. This study represents to our knowledge the first look at tetradeca in biosolids and will in the future be expanded to previously gathered samples through time to generate potential temporal distribution of this compound in Canadian WWTPs.

**WP095 Relationships of polybrominated diphenyl ethers with algal organic matter and fatty acids in sediment cores, South China**

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The effects of algal organic matter (AOM), fatty acids (FAs), and climate change on the deposition history of polybrominated diphenyl ethers (PBDEs) in the subtropical lake and reservoir sediment cores, South China were investigated. The sedimentary fluxes of PBDEs were estimated. The results show that the patterns of PBDEs are dominated by BDE-209, followed by BDE-47, BDE-99, and BDE-183. Algae make a dominant contribution to the sedimentary organic matter. The downcore contents of PBDEs and AOM in these cores show decreasing trends. AOM contents are significantly related to PBDEs in the cores, indicating the importance of algal productivity. Contents of FAs in the meso-eutrophic reservoir cores are significantly correlated with those of AOM and PBDEs, further confirming the role of algae to the transfer and deposition of PBDEs. Climate warming is observed to enhance the algal productivity in the reservoirs, thus elevating the deposition of PBDEs. Finally, the fluxes of PBDEs in the meso-eutrophic reservoir and the oligotrophic reservoir are estimated to be 2.22 ng/cm$^2$ yr and 0.959 ng/cm$^2$ yr, respectively, which increase with the increasing trophic level.

**WP096 Short-, Medium-, and Long-Chain Chlorinated Paraffins in Wildlife from Paddy Fields in the Yangtze River Delta**

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Short-chain chlorinated paraffins (SCCPs) were added to Annex A of the Stockholm Convention on Persistent Organic Pollutants in April, 2017. As a consequence of this regulation, increasing production and usage of alternatives, such as medium- and long-chain chlorinated paraffins (MCCPs and LCCPs, respectively), is expected. Little is known about the environmental fate and behavior of MCCPs and LCCPs. In the present study, SCCPs, MCCPs, and LCCPs were analyzed in nine wildlife species from paddy fields in the Yangtze River Delta, China, using atmospheric pressure chemical ionization-quadrupole time-of-flight mass spectrometry. SCCPs, MCCPs, and LCCPs were detected in all samples at concentrations ranging from < 91-43000, 96-33000, and 14-10000 ng/g lipid, respectively. Most species contained primarily MCCPs (on average 44%), with the exception of collared scops owl and common cuckoo, in which SCCPs (43%) accumulated to a significantly (i.e. $p< 0.05$) greater extent than MCCPs (40%). $Cl_6$ groups were dominant in most species except for yellow weasel and short-tailed mamushi, which contained primarily $Cl_7$ groups. Principal components analysis, together with CP concentrations and carbon stable isotope analysis showed that habitat and feeding habits were key factors driving CP accumulation and congener group patterns in wildlife.

### WP097 Organophosphate Ester Flame Retardants in San Francisco Bay

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As the largest estuary on the West Coast of the Americas, the San Francisco Bay drains approximately 40% of the state of California, and supports a dense local urban population. These features make the region an excellent laboratory for studies of anthropogenic contaminants. The Regional Monitoring Program for Water Quality in San Francisco Bay (RMP) supports strategic studies of contaminants of emerging concern (CECs) in the Bay, including monitoring of polybrominated diphenyl ether (PBDE) flame retardants and their alternatives. The results of a pilot study characterizing a broad array of flame retardants in multiple Bay matrices suggested organophosphate esters (OPEs) merit further study. OPEs are used as flame retardants and plastic additives and are water soluble. Some members of this class have predicted no effect concentrations (PNECs) less than 1 µg/L. Analysis of over a dozen OPEs in ambient Bay water samples collected in 2017 will be presented.

### WP098 Tissue-distribution and in-ovo maternal transfer of PBDEs and non-PBDE flame retardants in Great Lakes Herring Gulls

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Herring gulls (*Larus argentatus*) of the North American Great Lakes are primarily consumers of the aquatic ecosystem, but are also opportunistic and will feed on terrestrial diet items. Herring gulls have been a sentinel species for long-term contaminant monitoring for the past 40 years. Conventional techniques of avian monitoring rely on the measurement of whole (i.e. homogenized) eggs or pools. Generally there are few studies that have examined the extent of the tissue-specific distribution of chemicals of emerging environmental concern (CECs), and the extent of maternal transfer from mother birds to their offspring *in ovo*. This study investigated the tissue-specific (i.e. liver, adipose, muscle, blood (plasma and red blood cells), and brain) distribution of polybrominated diphenyl ethers (PBDEs) and several non-PBDE flame retardants (FRs) in mother herring gulls from Chantry Island, Lake Huron, and the extent of *in-ovo* transfer to their eggs (i.e. yolk and albumen). Comparisons were made to the distribution profiles of per- and polyfluorinated alkyl substances (PFASs) and organophosphate esters (OPEs) previously measured in the same maternal gulls and eggs. The predominant BDE congeners in both herring gull tissues and eggs were BDE-47, -99, -100, -153, -154, and -209, while Dechlorane Plus isomers and hexabromocyclododecane (HBCDD) were the principal non-PBDE FRs. Total PBDE concentrations were > 10x higher than combined non-PBDE FR concentrations in all tissues, while rates of maternal transfer were similar (6-7%) for each compound. PBDEs and HBCDD showed similar concentrations in both gull red blood cells (RBCs) and plasma, in comparison to OPEs and PFASs, which preferentially accumulated in the RBCs and plasma, respectively. The extent of maternal transfer was substantially greater for PFASs (~80%), while OPE transfer was intermediate of the PFASs and PBDE/non-PBDE FRs. This is also the first known report on the presence of methoxylated polybrominated diphenoxy benzene contaminants in an animal body compartment (i.e. liver, muscle, and adipose) in concordance with previously reported measurements in Great Lakes herring gull eggs. These results emphasize the importance of understanding the distribution and extent of maternal transfer of CECs for future monitoring. For example, the low extent of maternal transfer for more highly brominated compounds may indicate that relying solely on egg measurements may underestimate total body burdens of birds.

### WP099 Prioritization of 10 organic flame retardants using an avian hepatocyte toxicogenomic assay

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As the number of chemicals developed and used by industry increases, the inherent limitations of traditional toxicology approaches become an unavoidable issue. To help meet the demand for toxicity evaluation, new methods, such as high-throughput toxicity screening, are currently being developed to permit rapid determination of toxic, molecular and/or biochemical effects of a wide range of chemicals. In the present study, we demonstrate the utility of an avian in vitro toxicogenomics screening approach to determine the cytotoxic and transcriptomic effects of 10 organic flame retardants (OFRs) currently of international priority for ecological risk evaluation in order to prioritize and inform future toxicological studies. Hepatocytes from two avian species, chicken and double-crested cormorant, were prepared and exposed for 24 hours to various concentrations (0 to 300 µM) of OFRs among which were p-tert-Butylphenyl diphenyl phosphate (BPDP) and phenol, isopropylated, phosphate (3:1) (IPPP). Cell viability, the 7-ethoxyresorufin-*O*-deethylase (EROD) assay, and transcriptomic analysis using species-specific ToxChip PCR arrays were performed to evaluate the in vitro effect of these OFRs. Of the 10 OFRs assessed, BPDP and IPPP elicited the strongest cytotoxic and transcriptomic response in both chicken and double-crested cormorant hepatocytes and are therefore recommended as priority candidates for further toxicological investigations.

### WP100 Biomarkers responses in the fish *Prochilodus lineatus* after TCPP and TDCP exposure

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Organophosphate flame retardants (OPFR) are widely used, are found in the environment and accumulate by the biota. The aim of this study was to evaluate the effects of TCPP and TDCP in the neotropical fish *Prochilodus lineatus*. Juveniles were exposed for 96h to 1.5 and 30 µg/L of TDCP, to 2 and 200 µg/L of TCPP, and to a mixture of 1.5 µg/L of TDCP and 2 µg/L of TCPP. A control treatment was also run. Gills and liver were removed for analysis of Glutathione S-transferase (GST) and ethoxyresorufin-O-deethylase (EROD) activities, and lipid oxidation levels (LPO) and GSH content. No mortality was observed. No statistical difference was observed in EROD and GST activity in the gills between treatments. EROD in the liver of animals exposed to the mixture of OPFR was higher than those of the animals exposed to only TCPP or TDCP, but no difference occurred in relation to the control animals. GST activity was inhibited in the liver of animals exposed to the mixture of OPFR in relation to the animals exposed to 30 µg/L of TDCP and TCPP, but there was no difference in relation to control animals. GSH content in the liver was not altered by OPFR exposure, but it was elevated in the gills of the fish exposed to the mixture in relation to those exposed to TDCP and 200 µg/L of TCPP. No lipid damage was observed after OPFR exposure. These results indicate that some biomarkers can be altered by OPFR exposure and that the exposure to the mixture of TCPP and TDCP caused more alterations than the exposure to TCPP and TDCP separately. More studies are necessary to establish the mechanism of toxicity of TCPP and TDCP to fish

**WP101 Tris (1,3-dichloro-2-propyl) phosphate exposure during gastrula induces anemia in zebrafish embryos**

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Tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) is a high-production volume organophosphate flame retardant commonly detected within indoor dust following migration from end-use products. Therefore, the potential for TDCIPP-induced developmental toxicity raises concern for pregnant women and children exposed through inhalation and ingestion of TDCIPP-contaminated dust. Within early zebrafish development, initiation of exposure to TDCIPP at 0.75 h post-fertilization (hpf) results in epiboly delays by 6 hpf, dorsalization by 24 hpf, and adverse effects on mesoderm-derived tissues by 72 hpf. Therefore, as red blood cells (RBCs) are derived from the mesodermal germ layer, the objective of this study was to determine whether initiation of TDCIPP exposure at 0.75 hpf results in adverse effects on RBC abundance by 72 hpf. Embryos were exposed to vehicle (0.1% DMSO), 0.047 µM butafenacil (an herbicide and potent inducer of anemia in zebrafish embryos), or TDCIPP (1.56 or 3.12 µM) from 0.75-72 hpf or 10-72 hpf, and 72-hpf embryos were fixed and stained with *o*-dianisidine to quantify hemoglobin as a read-out for RBC abundance. Initiation of TDCIPP exposure at 0.75 hpf resulted in a concentration-dependent decrease in hemoglobin abundance in the absence of gross malformations. However, initiation of exposure at 10 hpf significantly decreased the magnitude of effect on hemoglobin abundance at 72 hpf relative to initiation of exposure at 0.75 hpf, suggesting that the effect of TDCIPP on RBCs was dependent on exposure during gastrulation (~5-10 hpf) – a critical stage of embryogenesis that includes germ layer (e.g., mesoderm) development. Overall, our findings demonstrate that TDCIPP exposure during early embryogenesis results in anemia by 72 hpf – a phenotype that may be associated with TDCIPP-induced disruption of normal germ layer development.

**WP102 mRNA-Sequencing Identifies Liver as a Potential Target Organ for Triphenyl Phosphate in Embryonic Zebrafish**

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Triphenyl phosphate (TPHP) is a commonly used organophosphate flame retardant and plasticizer in the United States. Over the past decade, there has been a marked increase in the use of TPHP due to the phase-out of certain brominated flame retardants. Using zebrafish as a model, previous studies have shown that TPHP exposure from 24 to 72 hours post fertilization (hpf) results in severe cardiac looping defects by 72 hpf – a phenotype that is dependent on exposure during pharyngula (24-48 hpf) and mitigated by pre-treatment with non-toxic concentrations of a pan-retinoic acid receptor (RAR) agonist (fenretinide). Therefore, the objectives of this study were to 1) rely on mRNA-sequencing to identify pathways before and after cardiac looping (30 and 48 hpf, respectively) that may be impacted following exposure to 10 µM TPHP from 24-48 hpf and 2) determine whether pre-treatment with 2 µM fenretinide from 24-30 hpf mitigates cardiotoxicity-related pathways within embryos exposed to TPHP from 30-48 hpf. Based on mRNA-sequencing, TPHP exposure from 24 to 30 hpf and 24 to 48 hpf significantly affected the abundance of 305 and 274 transcripts, respectively, relative to vehicle (0.1% DMSO) controls. In addition to minor effects on cardiotoxicity- and nephrotoxicity-related pathways, Ingenuity Pathway Analysis (IPA) of significantly affected transcripts from 30- and 48-hpf embryos revealed that hepatotoxicity-related pathways were strongly affected following exposure to TPHP alone. Moreover, while pre-treatment with fenretinide mitigated TPHP-induced cardiac looping defects at 72 hpf, IPA revealed that fenretinide was unable to block TPHP-induced effects on cardiotoxicity-, nephrotoxicity-, and hepatotoxicity-related pathways at 30 and 48 hpf. Overall, our mRNA-sequencing-based data suggest that, in addition to the heart, the embryonic liver may be highly susceptible to TPHP exposure during early development. Therefore, future research is needed to 1) confirm that TPHP exposure alters the normal trajectory of liver development (based on phenotypic data) within zebrafish embryos and 2) identify the mechanism of action that leads to potential TPHP-induced effects on hepatocytes within zebrafish and human cell-based systems.

**WP103 Tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) exposure during early-blastula alters the normal trajectory of zebrafish embryogenesis**

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Tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) is a high-production volume organophosphate flame retardant used around the world. Due to leaching from end-use products into indoor dust, concerns remain about the potential effects of TDCIPP on human populations, particularly during early development. Using zebrafish embryos, we previously showed that initiation of TDCIPP exposure at 0.75 h post fertilization (hpf) leads to defects in embryogenesis, marked by epiboly arrest (4-6 hpf) and dorsalization (24 hpf). Interestingly, at 24 hpf, TDCIPP-induced dorsalization phenocopied embryos exposed to the bone morphogenetic protein (BMP) pathway inhibitor dorsomorphin (DMP), suggesting that TDCIPP may have a similar target as DMP. Therefore, using a combination of mRNA sequencing, pharmacologic, and immunohistochemical strategies, our overall objectives were to investigate the impact of TDCIPP on BMP signaling and identify other potential targets and downstream effects. We first showed that embryos are most sensitive to TDCIPP-induced effects when exposed within early-blastula (2-3 hpf). We also found that pre-treatment with 4'-hydroxychalcone (a BMP agonist) significantly mitigated TDCIPP-induced deformities. However, phospho-Smad1/5/9 immunostaining revealed that DMP – but not TDCIPP – blocked normal BMP signaling gradient, suggesting that TDCIPP does not impact BMP signaling. Similarly, mRNA-sequencing data revealed that TDCIPP did not significantly affect transcripts involved in BMP signaling. Interestingly, at the beginning of segmentation (10-12 hpf), TDCIPP exposures resulted in a decrease in transcripts that regulate mesoderm differentiation (*tbx16, tbx6, tbx6l,* and *msgn1*). Indeed, Tbx16 immunostaining revealed that the mesoderm was disrupted in TDCIPP-treated embryos, resulting in less-developed mesoderm-derived structures (e.g., somites and notochord). We also found that TDCIPP affected development of red blood cells (which are also mesoderm-derived) and eye, with decrease in hemoglobin, eye area, eye pigmentation, and retinal neurons by 72 hpf. Overall, our results suggest that initiation of TDCIPP exposure during early-blastula alters the normal trajectory of early embryogenesis, leading to abnormal development of vital tissues and organs within the embryo. As early developmental processes are conserved across all vertebrates, our findings suggest that TDCIPP has the potential to induce similar effects following *in utero* exposure in mammals.

**WP104 Effects of a brominated organophosphate flame retardant on amphibians during their aquatic life stages**

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The transition from tadpole to frog (metamorphosis) and sex differentiation are tightly controlled by the endocrine systems including hormones. Exposure to even low concentrations of certain environmental contaminants can change normal hormone processes that can have severe effects on biological development and thus the production of healthy, reproductively capable individuals. We evaluated the sex-steroid and thyroid disrupting potential of a high-risk brominated organophosphate flame retardant identified on Canada's Chemicals Management Plan

(CMP) priority new substances list as a suspected endocrine receptor binder. We assessed the toxicity and tissue residues of the flame retardant, 2,2-bis-(bromomethyl)-3-bromo-1-propanol phosphate (multiple CAS: 19186-97-1, 134376-16-2, 36483-58-6; molecular formula: $C_{15}H_{24}Br_9O_4P$) in two Canadian native species, *Lithobates sylvaticus* and *L. pipiens*. This substance would be used in consumer products and primarily enter the environment via waste water treatment effluents and the application of biosolids. First, we conducted standard 96 h acute toxicity tests to establish lethal and sub-lethal acute effects. We then used this information to select sub-lethal concentrations for chronic exposures to *L. pipiens*. We determined the lethal concentration that resulted in 50% mortality and assessed for growth differences and malformations in hatchlings exposed to the flame retardant at concentrations up to 245 µg/L compared to controls. Finally, *L. pipiens* tadpoles were chronically exposed for 30-days to sub-lethal concentrations of this compound, and growth, developmental stage and sex differentiation were assessed. Our study supports the hazard profile risk assessment of a CMP3 priority new substance by providing some of the first aquatic vertebrate toxicity data for this compound.

**WP105 Environmentally relevant concentrations of Triphenyl Phosphate induced Gonadal Intersex and Changed Reproductive Behavior in Japanese Medaka**

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Triphenyl phosphate (TPhP) as a typical kind of organophosphorus flame retardants (OPFRs), has been frequently detected in surface water and wildlife. To evaluate the reproductive toxicity of TPhP for fish, we exposed Japanese Medaka (Oryzias latipes) from hatching to sexual maturity (100 dpf) at 134.1, 299.1, and 1429.5 ng/L, and it was found that TPhP could induce gonadal intersex of male medaka at the lowest concentration. Chasing time of male fish before mating was increased about 2-fold in 299.1 ng/L exposure group, and it was regulated by the ratio of estrogen and androgens. Successful mating rate was significantly reduced from 100% at control to 56.25% at 1429.5 ng/L. TPhP also decreased fertilization and hatching rates of eggs, and the lowest observable effective concentration (LOEC) of TPhP to significantly inhibit fertilization and hatching rates were 1429.5, and 299.1 ng/L, respectively. We first demonstrated that TPhP could cause problems in fish testicular development and reproductive behaviors at environmentally relevant concentrations.

## Fate and Effects of Chemicals from Diffuse Sources and Stormwater

**WP106 Investigation of hemolytic effects in coho salmon exposed to urban stormwater**

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Coho salmon are uniquely sensitive to urban stormwater, as demonstrated in the recurrent acute mortality phenomenon affecting coho in urban Puget Sound lowland streams. Symptoms include increased surfacing and loss of equilibrium prior to death, suggesting that cardio-respiratory distress may lead to the acute mortality. Chemical toxicity is implicated because abiotic factors in urban streams such as dissolved oxygen and temperature are within levels that are safe for fish. Although the causative agents in stormwater runoff remain unknown, oxidative stress may result from mixtures of numerous anthropogenically-sourced pollutants, such as metals, pesticides, and polyaromatic hydrocarbons. An oxidative stress mediated disturbance may be particularly damaging to circulating red blood cells, resulting in hemolysis. In the current study, we assessed indicators of intravascular hemolysis in juvenile coho exposed to highway runoff. Free hemoglobin released into the blood plasma due to acute and chronic hemolysis has been linked to numerous vascular abnormalities in humans and may therefore offer a potential pathway for cardio-respiratory distress in coho suffering from severe oxidative stress.

**WP107 Urban stormwater runoff causes cardiac injury in forage fish embryos**

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Non-point source pollution, including untreated urban runoff, is the foremost water quality threat to aquatic habitats throughout the United States. In the Pacific Northwest, the impacts of toxic stormwater have been extensively studied for Pacific salmon and their prey. However, comparatively little is known about the vulnerability of marine forage fish such as Pacific herring (*Clupea pallasii*) and surf smelt (*Hypomesus pretiosus*). These keystone species spawn adhesive eggs on intertidal and shallow sub-tidal substrates, in close proximity to land-based runoff. To examine the impacts of stormwater runoff on forage fish embryonic development, we exposed herring embryos to 0, 12, 25, or 50% stormwater runoff beginning just prior to the onset of a visible heartbeat (5 dpf) through hatching (11 dpf). Embryos exposed to stormwater runoff accumulated up to 74 ng/g total polycyclic aromatic hydrocarbons (PAHs) including high levels of phenanthrene, pyrene and fluoranthene. Consistent with PAH exposure, *cyp1a* mRNA levels were significantly upregulated in embryos exposed to at least 12% stormwater for 2 or more days. Stormwater exposure caused significant reductions in larval length and eye size, suggesting developmental delay or reduced growth. In addition, herring exposed to stormwater runoff exhibited cardiac injury including both functional (e.g., altered contractility) and morphological (e.g., decreased ventricle area and altered ventricle shape) heart defects. In summary, stormwater runoff increased *cyp1a* mRNA expression, reduced embryonic growth and induced cardiac injury in Pacific herring embryos. These effects are remarkably consistent with the cardiac injury phenotype observed in oil-exposed fish and could result in delayed adverse outcomes such as reduced cardiorespiratory fitness and subsequent mortality.

**WP108 Tire dust toxicity on aquatic invertebrates**

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Road runoff contributes in large part to the total pollutant budget of receiving waters. Besides combustion engine emissions, brake and tire wear are major contributors to traffic-related particulate matter. Tire particulate waste released into the environment per capita is estimated at 0.81 kg/year. Tire dust has a complex chemical composition and some of its components are water soluble. During rain events chemicals from tire dust can be leached into stormwater runoff. Coho salmon (*Oncorhynchus kisutch*) are acutely sensitive to chemicals that leach from tires. A laboratory study was conducted to compare the acute sensitivity of coho salmon to that of sensitive aquatic invertebrate, the crustacean *Ceriodaphnia dubia*. A tire dust mix was made by grinding nine different tires (new and used) from light duty vehicles. A 10 g/L tire leachate was produced using a closed recirculating system, allowing water to run through the tire dust mix for 24 h. The eluate was tested at 1%,10%, 20%,100% concentrations to assess the toxicity of tire dust on the survival of *C. dubia* at 48 h and on the reproduction after 8 days of exposure. Whereas tire leachate was acutely lethal to coho salmon at 0.1 g/L ($LC_{50}$), the $LC_{50}$ for *C. dubia* was approximately 10-fold higher. Reproductive endpoints were the most sensitive to tire mixture leachate. Although *C. dubia* is widely considered a highly sensitive organism, this study demonstrates the variability in organismal response to constituents of road runoff and highlights that relying on one single-species test may not be representative.

**WP109 Assessment and management of stormwater impacts on sediment recontamination: You don't need to measure everything, just the right things**

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Stormwater sources are difficult to understand because of the poor characterization of the irregular, event-driven inputs and the difficulty of managing diffuse sources of large volumes of runoff. The study objective is to develop methodologies to quantify the consequences of ongoing low-level sources on chemical concentrations, contaminant availability, and effects on biological receptors in surficial sediments. The study area was in Paleta Creek near Naval Base San Diego (NBSD), in California. Two storm-events were captured for particle size characterization and chemical analysis. Receiving and outfall waters collected using auto-samplers, which were triggered at each location during two different seasons. The samples were analyzed for a variety of metals, PAHs, and PCBs, as a function of particle size. Sediment traps and sediment cores were also collected from the Creek and subjected to bulk chemical analysis. The fractionated water and sediment samples were processed for metal extraction using the modified EPA method 3005A and 3050B, respectively, and were analyzed using ICP-MS and MERX-T. Persistent organic pollutants in water samples were Liquid-Liquid Extracted (LLE) using the modified EPA Method 3510C, while sediment was extracted by Pressurized Fluid Extraction (PFE with ASE 350) using the modified EPA Method 3545A. PAH analysis was performed on HPLC and PCBs on GC/MS. Results showed that storm-events were dominated by coarse particles initially most likely to lead to sediment recontamination in the near field of the receiving water (e.g., PAH, and Cd). Cu was associated to the dissolved and clay fraction, however the depositing loads were more influenced by resuspension and redistribution of sediment than stormwater. Data suggested that PAHs and PCBs, due to low bioavailability as determined with passive sampling and bioaccumulation testing, are not a strong contributor to sediment toxicity which appeared to be better correlated to the presence of pyrethroids in the traps. The study indicated that the size-segregated contaminant loads and simultaneous receiving water measurements were very helpful in relating the stormwater discharges to sediment recontamination. The particle associations in stormwater along with spatial distribution particularly in sediment traps, and less in the sediment cores, can identify contributing locations, effective remedial approaches, and help to propose best practices for stormwater and sediment management.

**WP110 Characterizing water quality background concentrations of aluminum, PCBs, and radioactivity on the arid Pajarito Plateau, New Mexico**

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If NPDES permit action levels are set near or below background concentrations, achieving compliance is difficult or impossible and might serve only to attempt to reduce naturally occurring constituents. In regard to a NPDES stormwater permit for Los Alamos National Laboratory (LANL), which is situated on the arid Pajarito Plateau near Santa Fe, New Mexico (NM), studies show that aluminum and gross alpha radiation concentrations are attributable to Bandelier tuff, the major geologic media in the area. Meanwhile, atmospheric deposition of polychlorinated biphenyls (PCBs) contributes to an anthropogenic baseline, measurable in reference watersheds. As a result, exceedances of NPDES permit action levels and state water quality criteria (WQC) for these constituents are erroneously attributed to LANL discharges. To address this situation, a framework was established to generate water quality background threshold values (BTVs) that characterize natural background (NBG), anthropogenic baseline, and developed background conditions using statistical methods. Water quality data were evaluated for potential spatial and temporal dependencies, as well as relationships with suspended sediment concentration (SSC), a parameter positively correlated with storm water runoff. Data were stratified or normalized to SSC to address dependencies prior to calculating BTVs for 18 constituents, resulting in a total of 43 BTVs. A subset of those BTVs are discussed herein. Most BTVs were calculated using ProUCL as 95-95 UTLs based on gamma, lognormal, normal, or nonparametric methods. The resulting anthropogenic baseline PCB BTV of 0.058 µg/L is 90 times higher than the NM human health WQC (0.00064 µg/L). The NBG gross alpha BTV is 190 pCi/g SSC, which, after back-transformation using the 25$^{th}$ and 75$^{th}$ percentile of NBG SSC, ranges from 170 to 1,900 pCi/L, well above the NM WQC (15 pCi/L). Two NBG aluminum BTVs were developed (for subareas of Pajarito Plateau) of 17 and 76 mg/g SSC, which, after back-transformation, range from 15 to 1,700 mg/L and 68 to 78 mg/L, respectively; these values are much higher than the NM hardness-based WQC (ranging from 0.37 to 1.9 mg/L as total aluminum). These results suggest that BTVs should be taken into account before concluding that exceedances of state WQC and related LANL NPDES permit action levels are attributable to LANL discharges, leading to unwarranted actions such as engineered controls, 303(d) listings, and developing TMDLs.

## Alternative Animal Ecotoxicity Testing: New and Novel Approaches for Predicting Environmental Hazards and Risk Assessment

**WP111 Evaluation of Cu and CuO nanoparticle environmental impacts using laboratory small-scale microcosms**

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Copper based nanoparticles (NPs) are widely used in industrial and commercial products as sensors (49%), catalysts (20%), surfactants (6%), antimicrobials (4%), and for other purposes (21%). The high production volume and increasing use of copper-based NPs make the ecological risk of metallic copper or copper oxide (Cu and CuO) NPs a concern. The physico-chemical properties of copper-based NPs can impact their dissolution and aggregation behavior with concomitant impacts on their environmental toxicity. Here, we investigated NP dissolution, organismal uptake and aquatic toxicity of Cu, CuO NPs at 0, 0.1, 1, 5 or 10 mg Cu/L using a previously developed 5-day multi-species microcosm comprised of algae (*C. reinhardtti*), bacteria (*E. coli*), crustaceans (*D. magna*), and zebrafish (*D. rerio*). We hypothesized that Cu and CuO NPs can elicit differential toxicity to the organisms due to alterations in the bioavailability of dissolved copper species and variations in organismal nanoparticle uptake. The actual concentrations of dissolved Cu released from the NPs were compared to $CuCl_2$ ionic controls at the same concentrations to determine the relative contribution of particulate and dissolved Cu on organism uptake and toxicity. We found that both NPs had higher uptake in *D. magna* and zebrafish than equivalent ionic exposures, suggesting that both Cu-based NPs are taken up by organisms. Cu NP exposures significantly inhibited algal growth rate, *D. magna* survival, and zebrafish hatching while exposure to equivalent concentrations of $CuCl_2$ and CuO NPs did not. This indicates that Cu NPs themselves likely elicited particle-specific toxicity to the tested organisms. The increased potential of Cu relative to CuO NPs to generate reactive oxygen species could have contributed to the differential toxicity observed. Overall, this work was the first study to utilize a rapid assay design to evaluate the fate and eco-toxicological impacts of Cu and CuO NPs in an aquatic community.

**WP112 Inflammation of gill epithelia in fish causes increased permeation of polar organic chemicals via disruption of tight junctions**

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In fish, the gill epithelial layer controls paracellular permeation of chemicals through epithelial tight junctions. Previous research has indicated that the integrity of epithelial tight junctions can be adversely impacted by gill inflammation. A loss of junction integrity could lead to greater uptake of harmful contaminants, effectively increasing their toxicity. In this study we investigated if inflammation could induce disruption of tight junctions in fish gill epithelia, and if this in turn increases passive transport of chemicals across the epithelia. Inflammation was experimentally induced in a permanent rainbow trout gill cell line (RTgill-W1) through exposure to non-cytotoxic concentrations of lipopolysaccharide (LPS). Transepithelial electrical resistance (TEER) was used to indicate epithelial tight junction integrity. Cells were also co-exposed to LPS and oil sands process-affected water (OSPW) to determine if the hypothesized reduction in tight junction integrity would result in greater transport of OSPW across the gill cell epithelial layer in vitro. Quantitative real-time PCR (qPCR) was carried out to characterize changes in transcript abundance of genes responsible for tight junction proteins (e.g. claudins). Cells exposed to LPS showed significant reduction in TEER after 24 h of exposure. qPCR data showed that the abundance of transcripts of genes coding for tight junction proteins (i.e. Claudin 28b and 10e) were significantly decreased in cells exposed to 20, 50, and 100 mg/L LPS. Furthermore, chemical analyses revealed a significant increase in transport of constituents of OSPW across the gill cell epithelial layer at all concentrations of LPS. These in vitro findings were confirmed by *a*n in vivo exposure experiment with fingerling rainbow trout that showed a comparable increase in OSPW content in fish exposed to both LPS and OSPW for 48 h, compared to fish exposed to OSPW alone. These results indicate that fish living in environments high in pathogens could be at risk of greater uptake of contaminants of environmental concern (i.e. chemicals in OSPW) than previously thought. This in turn highlights the need to further assess the risks that exposure to pathogens may pose from a toxicological perspective.

**WP113 Ecological Threshold for Toxicological Concern (eco-TTC) – Applications for Environmental Risk Assessment in Various Contexts**

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The Threshold for Toxicological Concern (TTC) is well-established for assessing human safety but has only recently been explored in the ecological context. Ecological TTCs (eco-TTCs) summarize the wealth of ecotoxicological information as Predicted No-Observed Effect Concentrations (PNECs) on diverse chemical substances in the form of probability distributions. These enable the prediction of untested chemicals based on a structural attribute, mode of action, or functional use. The approach may be useful to assess chemicals at early tiers of the risk assessment process, providing hazard perspective on chemicals that lack QSARs, guiding product development discussions, and assisting read across or category justifications. A database was developed based on recent assessments of published data and international chemical management programs. This data is associated with physical chemistry data and curated taxonomic information for the organisms tested, including a process to conclude acute and chronic effects as well as identify the PNEC for exposed ecosystems based on depth and breadth of data. Several mode of action schemes are also included to facilitate development of a best approach for grouping compounds. To make these data accessible and useful, the dataset was transitioned into a MySQL format, allowing for a format that is relational and scalable, facilitating easy access, sharing, and integration with other datasets and tools. The dataset is accessed via a web-based query system that is integrated with a PNEC calculator and probability distribution tools. The interface allows users to explore the data, upload additional datasets, derive threshold values based on specific criteria, and explore use and application of the eco-TTC concept. A workshop was held to discuss and evaluate the feasibility of the approach, including evaluation of several case-studies based on particular decision-contexts (e.g., prioritization and screening, chemical risk assessment, site specific risk assessment, mixtures, product development, criteria development). The discussions and conclusions will be presented, including exploration of how this approach could be applied and integrated into evaluation strategies. *The views, conclusions and recommendations expressed in this article are those of the author and do not necessarily represent views or policies of the European Commission, Environment and Climate Change Canada, or the U.S. Environmental Protection Agency.*

**WP114 Estrogenic effects of thyroid hormone disruption in zebrafish**

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Thyroid hormones have been implicated in many physiological actions, such as metabolism, development, growth, and reproduction. The influences of thyroid hormone in gonadal development has been intensively studied in rodent species, and recently several studies in fish have highlighted the effect of reproduction by thyroid hormone disruption. We investigated the reproductive effects by thyroid hormone disruption in transcriptomic levels. Exposure of 3,5,3'-triiodo-l-thyroxine (T3, 1 nM) or propylthiouracil (PTU, 10 uM) to zebrafish induced estrogenic effect by regulating some key genes involved in steroidogenesis (estrogen production) and progesterone-mediated oocyte maturation pathway, including estrogen receptor (esr1 and esr2b) and vitellogenin (vtg1 and vtg2). by analyzing these molecular biomarkes, is information, suspected thyroid hormone disrupting chemicals, bisphenol S (BPS), tris (1,3-dichloro-2-propyl) phosphate (TDCPP), and di-(2-ethylhexyl)-phthalate (MEHP) were evaluated in the reproductive effects and found out that only MEHP showed distinct changes in these gene expression, which might be from thyroid hormone disruption. However it is necessary to evaluate qualitative and quantitative evidence of thyroid hormone disruption in zebrafish prior to conclude the association of thyroid hormone disruption caused by MEHP with the estrogenic effects. This study could provide important information on the reproductive toxicity caused by internal thyroid hormone disruption and also contribute to find novel biomarker(s) for screening estrogenic effect by chemicals that could cause thyroid hormone disruption.

**WP115 Application of Adverse Outcome Pathways to Non-Chemical Stressors: Examples assessing Sea Lamprey Parasitism and Warmed Winter Water Temperatures**

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The adverse outcome pathway (AOP) concept is designed to describe a mechanistic-based progression from a molecular initiating event to an adverse outcome based on a perturbation from a chemical stressor. Although the AOP framework has gained considerable traction among toxicologists, its acceptance and use is limited. The inclusion of non-chemical stressors into the AOP framework has potential to broaden its applicability by creating a unified system through which interactions between chemical-based and ecology-based stressors can be assessed. This integrated system may improve the robustness of AOP networks and add credibility to adverse outcome pathway-based regulatory decision making and risk assessment. Non-chemical stressor based AOP development will require several small changes to the AOP framework such as allowing for initiating events at non-molecular levels of organization. We illustrate potential avenues for AOP development by incorporating two non-chemical stressors that include mechanical and thermal stressors. The first describes how epithelium loss from sea lamprey (*Petromyzon marinus*) parasitism leads to decreased sperm quantity and quality in a host lake trout (Salvelinus namaycush). The second describes how warmed winter water temperatures initiate early reproductive development leading to reproductive dysfunction during the normal spawning period. Expanding the AOP framework to include non-chemical stressors may improve AOP network development, contribute to quantitative AOP development, and will entice other disciplines to be involved and apply the AOP framework to their study systems.

**WP116 Development of a Novel Cell-Based Model for Assessing Potential Toxicity of Seafood Through Bioanalytical Tools**

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The health benefits of a diet high in fish are well-known, but unfortunately there are also significant health concerns associated with eating fish exposed to a variety of contaminants. Methylmercury, PCBs, and Dioxins are currently considered by many states for seafood consumption advisories; however, there is little known about the risks of many other chemicals now routinely detected in fish. With a growing need to reevaluate the compounds being considered in fish consumption risk assessments and subsequent consumption advisories, the need for a method of determining mechanisms of toxicity associated with seafood consumption is ever-present. The current study utilizes in vitro systems to model the human dietary pathway in a co-culture model to facilitate the identification of contaminants driving adverse biological responses. To reproduce the complexity and cellular interplay that occurs in the digestive system, the current study uses an intestinal cell model, the human adenocarcinoma Caco-2 cell line that spontaneously differentiates in vitro, expressing several morphological and functional characteristics of mature small intestinal enterocytes. For the liver, human HepaRG cells were used, which can undergo a complete hepatocyte differentiation program. HepaRG cells express high functional levels of most of the major xenobiotic metabolizing cytochrome P450s (CYPs), most of which are poorly expressed or not inducible in other liver cell lines. Intestinal Caco-2 cells were cultured on a transwell insert, with hepatic HepaRG cells cultured underneath, replicating the natural flow in digestive movement, and allowing for metabolic activity to take place. Chemical extractions were conducted utilizing fish and oyster standard reference material (SRM) from the National Institute of Standards and Technology (NIST). Dosing of this extract to the digestive co-culture model has shown cytotoxic effects, and further analysis of CYP induction and quantification of oxidative stress is described. These bioanalytical tools are used to identifying significant biological responses that are outside the predicted effect level of current screening advisories, and can help in supporting revisions of current regulations to protect human health.

**WP117 Quantitative ability of the CALUX assay for simple environmental extracts: A case study with estrone-spiked pond water**

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Chemically-activated luciferase expression (CALUX) cell bioassays are relatively inexpensive, internationally utilized screening tools for various types of environmental pollutants such as dioxin-like chemicals (DLCs) or endocrine active substances (EAS). The dioxin CALUX cell bioassay is one of two U.S. Environmental Protection Agency-approved methods for quantifying levels of DLCs in many different sample types (the other method being gas chromatography-high resolution mass spectrometry [GC-HRMS]). However, CALUX assays are more problematic for quantifying levels of EAS due to the greater complexity of endocrine-active pathways in comparison to the well-characterized biochemical pathway and chemistry for DLCs. Nevertheless, many studies report estimates of ‘estrogen equivalents’ (EEQs) in complex samples and may compare these values to levels of estrogens detected by analytical chemistry methods (such as liquid chromatography quantitative time-of-flight MS [LC-QTOF-MS]). For complex samples such as environmental water or sediment, the EEQ values obtained by LC/MS often underestimate those generated by EAS assays. For simple samples, however, we explored the suitability of estrogen CALUX bioassays for quantitative purposes in water samples from laboratory studies. A series of control, estrone (E1), and E1/atrazine-spiked water samples for an in vivo fish exposure study was quantified with LC-QTOF-MS and analyzed with two estrogen CALUX cell bioassays, the OECD-approved VM7Luc4E2 cell bioassay and the VM7LucERbc9 cell bioassay (which is the VM7Luc4E2 cell line stably transfected with estrogen receptor beta). A method for screening environmental samples in 384-well plates was established in our laboratory for both of the estrogen CALUX cell bioassays with minimum detection limit (MDL) and half-maximal activity ($EC_{50}$) values of approximately 0.02-0.07 pg E1 and 0.24-0.28 pg E1, respectively. Concentrations of E1 in water as determined by LC-QTOF-MS were approximately 1 ng/L in the control tanks, 10-100 ng/L in the dosed tanks, and 10 mg/L in spiked water. Levels of E1 in water samples were then compared to those generated by the estrogen CALUX assays. This information can be used to strengthen laboratory exposure studies, demonstrating further utility of the CALUX assays.

**WP118 EcoToxChip: A toxicogenomics tool for chemical prioritization and environmental management (24 month update)**

*N. Basu, McGill University / Faculty of Agricultural and Environmental Sciences; M. Hecker, University of Saskatchewan / School of the Environment & Sustainability and Toxicology Centre; D. Crump, Environment and Climate Change Canada / National Wildlife Research Centre; J. Head, McGill University / Natural Resource Sciences; N.S. Hogan, University of Saskatchewan / Department of Animal and Poultry Science and Toxicology Centre; J. Xia, McGill University; G. Hickey, McGill University / Macdonald Campus / Natural Resource Sciences; S. Maguire, McGill University / Desautels Faculty of Management*

Our Large-Scale Applied Research Program (LSARP) grant from Genome Canada aims to develop, test, validate, and commercialize quantitative PCR arrays (EcoToxChips) and a data evaluation tool (EcoToxXplorer.ca) for the characterization, prioritization, and management of environmental chemicals and complex mixtures of regulatory concern. In Project Phase 1, EcoToxChips will be developed for laboratory model species representing the most important vertebrate groups in ecological risk assessment (fish-fathead minnow; bird-Japanese quail; amphibian-*Xenopus laevis*). Model species (adult and early-life stage, ELS) will be exposed via standardized tests to 8 environmental chemicals representative of natural resource/environment sectors of Canadian concern and also ones that impact a wide biological space (EE2, chlorpyrifos, benzo(a)pyrene, lead, fluoxetine, selenomethionine, trenbolone, HBCD) (Activity 1). An integrative systems approach based on functional ‘omics (combined global transcriptomic and proteomic profiling, targeted metabolome) and physiological analyses across levels of biological organization will then be applied to characterize relevant toxicity pathways including adverse outcome pathways, AOPs (Activity 2); from this, and other resources, species-specific EcoToxChips consisting of 384 environmentally-responsive genes of regulatory concern will be informed, built, tested, and optimized (Activity 3). EcoToxChip performance will be validated (and further optimized) through an inter-lab study with our collaborators (Activity 4). Under Activities 5-7, knowledge from Phase 1 will be translated to 3 native species (i.e., fish: rainbow trout; bird: double-crested cormorant; amphibian: wood frog). EcoToxXplorer.ca will provide intuitive bioinformatics support and be modeled on our successful cloud-based tools (metaboanalyst.ca). To position the team advantageously with regard to the commercialization and institutionalization of the deliverables, our GE3LS research will produce and leverage social science knowledge about the phenomenon of "institutional entrepreneurship". The anticipated socioeconomic benefits associated with the adoption of our deliverables, namely more focused animal testing, improved regulatory decision-making, and cost-efficiencies. Here we provide a 24 month update of our project (www.ecotoxchip.ca).

**WP119 Design thinking to inform micro-level features of the EcoToxChips and EcoToxXplorer.ca**

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Our Large-Scale Applied Research Program (LSARP) grant from Genome Canada aims to develop, test, validate, and commercialize quantitative PCR arrays (EcoToxChips) and a data evaluation tool (EcoToxXplorer.ca) for the characterization, prioritization, and management of environmental chemicals and complex mixtures of regulatory concern. To help ensure that project deliverables are adopted by end-users, the objective of this work was to incorporate design thinking into the development of the EcoToxChips and EcoToxXplorer.ca. This product design methodology includes 6 steps: 1) Collect data to understand users; 2) Determine how to address users' issues; 3) Generate product ideas; 4) Build product prototypes; 5) Test prototypes; and, 6) Make product. Key activities, outputs, and outcomes were discussed as they pertain to "micro-level" design of the project deliverables. First, a process was established to solicit information from user groups as to the relevance and importance of specific design features. This activity resulted in a 'registry' within which information from 26 informants has been collated to date. Second, with key insights extracted from each interviewee, an inventory of use cases (chemical screening and prioritization; hazard identification/MOA; environmental monitoring) and specific user-journeys (evaluating new chemicals; industry R&D; Great Lakes monitoring) were built to help focus the design of our deliverables. Third, a divergent and convergent phase ensued to help narrow down a set of deliverable features and detail how they may be realized. Finally, prototypes of the EcoToxChip (e.g., 384 well plate with ~30 QC genes and ~350 target genes organized into ~25 toxicity modules, etc…) and the EcoToxXplorer.ca (e.g., cloud-based tool with 3 main components being data upload and QC, statistical and functional analysis, report generation) were developed, and are now being deliberated upon by project team members, collaborators and end-users. To leverage iterative consultations with stakeholders, the most updated prototypes will be presented at SETAC for the at-large community to comment upon, before we launch manufacturing of the EcoToxChips, and continue programming of the EcoToxXplorer.ca.

**WP120 BE-SPME as a predictive screening tool for exposure to petroleum substances in the fish embryo toxicity test (OECD 236)**

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The success of the OECD 236 fish embryo test suggests potential utility in animal alternative testing. The objectives of the present study were to characterize the sensitivity of sublethal endpoints in the OECD 236 tests relative to other aquatic species following exposures to petroleum substances. A biomimetic solid-phase microextraction method was employed to measure total bioavailable hydrocarbons as a common exposure metric across compositionally distinct test substances. Results indicate that sublethal endpoints occurred just prior to lethality, though no one phenotype was consistently more sensitive than another. Additionally, whole transcriptome microarray was used to observe the transcriptome profile of developing zebrafish in response to similarly classed test substances. The transcriptome profile was compared amongst test substances as well as between test substance treatments. Using the sublethal morphological threshold established prior, we explored the transcriptome profile of embryos which displayed morphological alterations and embryos which appear to be normal, providing insight into limitations of biological response which precede morphological alterations.

**WP121 Kinetics of Glutathione Depletion and Antioxidant Gene Expression as Indicators of Chemical Modes of Action Assessed in Two In Vitro Models**

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Here we report a vertically integrated in vitro – in silico study that used seven diverse chemicals (cumene hydroperoxide, t-butyl hydroperoxide, hydroquinone, t-butyl hydroquinone, Bisphenol A, Dinoseb, and peflourooctanoic acid) suspected of inducing OS to probe the relationship between chemical properties, antioxidant gene expression, glutathione (GSH) depletion, and viability effects. Concentration-depend viability effects were assessed by MTT assay in two Hepa-1 liver cell line: a control cell line and a transfected line with increased glutamate-cysteine ligase (GCL) activity and GSH content. Change in of *Gclc*, G*clm*, *Hmox*, and *Nqo1* gene expression and intracellular GSH content in response to sublethal chemical exposure in both cell lines were monitored by RT-qPCR and naphthalene dicarboxaldehyde (NDA) derivatization, respectively. Peroxides and quinones had the largest effects on antioxidant genes expression at 6 or 24 h after exposure and only quinones caused significant GSH depletion. Overall the transfected cell lines were more resistant to chemical toxicity and showed marked decrease in OS biomarkers. The differences in viability effects were largest for peroxides and quinones and absent for Dinoseb. Finally, the temporal expression pattern of *Nqo1* gene differed from other antioxidant genes and was largely affected by quinone exposure. Given the biological data and known multifaceted biological function of quinones, the contributions of redox cycling and arylation to cytotoxicity and GSH depletion were evaluated computationally. Model results agreed with biological observations and indicated that t-butyl hydroperoxide produced ROS more readily than hydroquinone, while hydroquinone is more reactive towards GSH and other intracellular nucleophiles. The results highlight the importance of modes of action and molecular initiating events in chemical toxicity and the vital role of GSH in peroxide and quinone metabolism in vitro. Furthermore, the work emphasizes the opportunities presented by computational chemistry in elucidating modes of action and assessing chemical toxicity.

**WP122 Acute and Chronic Effects of Sodium Chloride on 3 Fish Cell Lines and Their Suitability as in vitro Alternatives**

*T.R. Bouchard, Lakehead University / Biology; P. Lee, Lakehead University*

Millions of animals are used annually for regulatory testing worldwide. Recent changes in government regulations have led to an increased need to find suitable alternatives for animals in testing. One potential alternative is the use of fish cell lines for in vitro toxicity tests. Fish cell lines have been studied as an in vitro alternative as they are derived from the organisms being replaced. In this study we evaluated the suitability of three fish cell lines as in vitro alternatives for acute and chronic toxicity testing. The study utilized the RTL-W1, RTGill-W1, and FHML cell lines and evaluated their responsiveness to acute and chronic exposures to NaCl using a modified crystal violet assay. The cell lines were plated in 96-well plates and then exposed to a solution containing L-15media spiked with NaCl. Exposure periods varied from 24hrs to 48hrs for acute and chronic tests respectively. Exposed cells were stained with crystal violet and their absorbance was measured at 570nm. $EC_{50}$ and $IC_{25}$ values were calculated using the absorbance values and compared to in vivo literature values acquired from the ECOTOX online database. The RTGill-W1 $EC_{50}$ value of 20.02g/L was found to not be significantly different from the Rainbow trout literature value of 8.92 g/L, while the RTL-W1 $EC_{50}$ value of 1.026 g/L was found to be significantly different from the literature value. The FHML cell line $EC_{50}$ value of 16.27 g/L was found to not be significantly different from the literature value of 7.25 g/L. Due to limited chronic literature data involving rainbow trout, $IC_{25}$ values from all three cell lines were compared to literature values for fathead minnows. The RTGill-W1 cell line had an $IC_{25}$ of 5.77 g/L, the RTL-W1 had an $IC_{25}$ of 1.19g/L, and the FHML cell line had an $IC_{25}$ value of 1.51 g/L and none of these values were found to be significantly different from the literature value of 1.72 g/L. These results demonstrate the potential for cell lines to be used for acute and chronic in *vitro* testing. Further studies will work to confirm the results of these tests, as well as to determine if this method can be applied to other toxicants and real world samples.

**WP123 F2-Isoprostanes in fish mucus: A new, non-invasive analysis of oxidative stress biomarkers**

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Prostaglandin-$F_2$-like derivatives, namely the F2 isoprostanes (F2-isoPs), have emerged as a reliable biomarker class for oxidative stress in mammals and fish. F2-isoPs are the free-radical catalyzed products of non-enzymatic lipid peroxidation of arachidonic acid, a fatty acid found in brain tissue and cell membranes. Fish mucus has been investigated in several studies as a potential biological matrix for the analysis of oxidative stress, as it is minimally invasive. It is composed mainly of glycoproteins, but notably contains immunoglobulins, pheromones, lysozyme and proteolytic enzymes. Mucus is known to have important biological functions for fish, ranging from communication and reproduction to osmotic regulation. To date, no method for the isolation and quantification of F2-isoPs in fish mucus has been reported. The aims of this study was to develop an efficient method for the extraction of F2-isoPs from fish skin mucus and to optimize the resolution and quantification of F2-isoPs by high-performance liquid chromatography tandem mass spectrometry. The method was based on an extraction of mucus with methanol under acid conditions. The extract was then centrifuged, filtered, and concentrated. Separations were performed on C18 (2.1mm x 50mm, 3.5µm particle size) column using methanol (0.1% formic acid) and water as the mobile phase. Negative electrospray ionization and specific multiple reaction monitoring ion transitions were used to detect F2-isoPs in mucus. Mass labelled internal standards were used to monitor recovery of native compounds during sample work-up and also to quantify native F2-isoPs. Native isomers of the Class III and VI F2-isoPs were measurable in Northern Pike (*Esox lucius*), lake trout (*Salvelinus namaycush*), and small mouth bass (*Micropterus dolomieui*). This work demonstrates that mucus has the potential to be used as a non-invasive, non-lethal matrix for F2-isoPs analysis in fish.

**WP124 Development of methods for the assessment of vision and neurological function in larval fathead minnows**

*J. Krzykwa, K. Roush, M.K. Sellin Jeffries, Texas Christian University / Department of Biology*

Recent efforts have begun to expand the zebrafish fish embryo toxicity (FET) test to other test species, including the fathead minnow. While studies have suggested that the zebrafish FET test method is transferable to the fathead minnow, there is interest in modifying the fathead minnow FET test to allow for estimation of sublethal adverse effects so that it could be used as a potential replacement for not only acute tests, but also chronic tests. To assess sublethal adverse effects, appropriate sublethal FET test endpoints must first be identified, then their predicative ability must be assessed. Previous studies have identified embryonic eye size as a potential FET test endpoint, and though there is limited evidence suggesting that these alterations are indicative of altered neurological development, studies validating the link between eye size and organism fitness are needed. The overarching goal of this project is to investigate whether reduced embryonic eye size at the conclusion of the FET test is predicative of altered vision or neurological function in larval fathead minnows. However, assays must first be developed to assess of vision and neurological function in larval fathead minnows. Therefore, the objective of the present study was to develop methods to assess vision/neurological function in larval fathead minnows. One method utilized to evaluate vision in fish is the assessment of optomotor response (OMR), an instinctual response in which a fish follows a moving reference point. Factors such as at what age fish reliably exhibited OMR, the necessary acclimation time, and the relationship between larval size and OMR were evaluated. The results of this study indicate that larval fathead minnows exhibit detectable OMR starting at 9 days post fertilization (dpf) and 4 min of acclimation to the chamber is needed. Interestingly, there is a significant relationship between length and OMR response, suggesting that larval size should be taken into account in future studies utilizing OMR as a metric. These results will be utilized in future studies investigating whether reductions in embryonic eye size are predictive of sublethal adverse effects and can also be utilized by other researchers interested in assessing vision/neurological function in larval fathead minnows.

## Sustainability and Ecosystem Services: From Science to Societal Choices

**WP125 Determinants of participating in the Deepwater Horizon oil spill cleanup**

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The *Deepwater Horizon* oil rig spilled 4.9 million barrels of oil in the Gulf of Mexico on April 20, 2010. An environmental disaster of such magnitude has detrimental effects on people living in affected areas; engagement of community members in recovery efforts is important for mitigating some adverse effects of oil spills. Few studies have explored factors that determine participation in oil spill cleanups, motivating this study. Publicly accessible data from the Gulf States Population Survey (GSPS) were analyzed to study the determinants of participation in the Gulf oil spill cleanup. Conducted by the Centers for Disease Control and Prevention from December 2010 to December 2011, the GSPS was a telephone survey of 38,361 adults in counties and parishes in Alabama, Florida, Louisiana, and Mississippi impacted by the oil spill. We used survey estimation to account for the complex survey study design and used multiple imputation by chained equations to handle missing data. We estimated the probability of participating in the cleanup among demographic subgroups and used univariate logistic regression models to test whether demographic factors were associated with participation. Approximately 4.6% (95% confidence interval [CI]: 3.7-5.6) of residents in these communities participated in the oil spill cleanup. Participants in cleanup efforts were mostly aged 25-34 years (26.8%; 95% CI: 17.7-35.8), men (56.1%; 95% CI: 45.7-66.5), non-Hispanic white (68.1%; 95% CI: 57.4-78.9), and employed at the time of the survey (64.2%; 95% CI: 53.3-75.1). Many cleanup participants (65.0%; 95% CI: 54.6-75.4) reported very good or excellent overall health at the time of the survey. Different frequencies of specific cleanup tasks were reported: over 70% (95% CI: 61.8-80.5) of cleanup participants assisted with beach or marsh cleanup, while only 3.7% (95% CI: 0.51-6.9) participated in well-head cleanup or controlled burning. Logistic regression analyses indicated that living in an affected coastal county was significantly associated with higher odds of participation in a cleanup (unadjusted odds ratio [OR]: 1.70; 95% CI: 1.29-2.25), as was having excellent or very good physical health (OR: 2.08; 95% CI: 1.14-3.77). Older persons were less likely to participate in the cleanup (OR for 65+ age group vs. 18-24 age group: 0.14; 95% CI: 0.05-0.35). Understanding the demographics of cleanup participants may help future oil spill responses determine how best to recruit community members in this region.

**WP126 Assessing the potential opportunities and tradeoffs of algal growth from wastewater**

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Algae holds much promise as a potential feedstock for biofuels, but large-scale algal biomass production has not yet demonstrated the performance required for the economical production of biofuels. To date, the biofuel industry has focused on algal monocultures- single species of algae that have high growth rates and lipid accumulation potentials when cultured under controlled laboratory conditions. However, scaling up monocultural production has remained a significant challenge because monocultures are extremely susceptible to pests and disease. Further, nutrient and water supplies will become limiting as we scale up production unless new approaches are developed to mitigate these resource constraints. The use of wastewater resources that are rich in nitrogen and phosphorus to supplement a portion, or completely replace, traditional fertilizer and water requirements may help significantly reduce algal production costs and conserve freshwater resources. Here we examine the performance of monoculture and polyculture algal crops in two case studies: municipal wastewater, and the waste from biofuel production. We examine the economic and environmental benefits, tradeoffs, and scalability of each of these case studies. Our results suggest that cultivating and managing algal polycultures in wastewater may be a more sustainable and cost-effective solution for the biofuel industry than the current practice of monoculture production.

## Recent Advances in Training, Online Support and Responsible Management Practices for LCA Databases

**WP127 Bringing it all together: Green Design and Assessment (GDA) Workbook**

*A. Nestler, A. Baker, L. Heine, Northwest Green Chemistry*

In order to design products for the circular economy, designers must complete an assessment of key product sustainability attributes at the earliest stage possible. This is essential to avoiding the sunk cost effect and providing ample time to redirect efforts if terminal problems are discovered. Designers understandably struggle to weigh tradeoffs. It's challenging to compare GHG emissions to toxicity, ethical working conditions to cost, or hazardous materials to performance. Guidance for making such decisions is the groundwork needed to usher in a functional circular economy. Northwest Green Chemistry (NGC) created the GDA Workbook, which is free and publicly available online, to help entrepreneurial product designers overcome this barrier. It is rooted in three overarching green chemistry and engineering principles: preserve natural capital, eliminate toxics in products & processes, and design holistically using life-cycle thinking. It informs early-stage assessment and empowers designers to continually improve products. This presentation will include an overview of the workbook with case studies. NGC collaborated with diverse stakeholders to better understand what businesses need to effectively evaluate and optimize product sustainability. An overarching theme was the desire for simple checklist-style guidance to evaluate whole products, not just individual chemicals. Businesses need a way to synthesize chemical hazard assessment, exposure assessment, and life cycle assessment (LCA) into a single, multifaceted evaluation. The Workbook enables this by benchmarking each life cycle stage against sustainable design principles using currently available tools. The GDA Workbook is designed to be accessible for those with varying expertise in sustainability, offering screening and advanced modules, and helping bridge the communications gap between sustainability scientists, designers, engineers, managers, and the public. It can help resource-strapped designers pinpoint problem areas and determine in which technical assessments, like formal LCA, to invest. This proactive approach can be applied by any product or process designer to optimize the sustainability of their product while avoiding unexpected trade-offs or costs. This approach creates an entry point into the complex world of sustainability and the circular economy. The interactive tool offers unique support by aligning tools with principles in support of a resilient circular economy.

**WP128 Data Modalities and Institutional Roles in Life Cycle Inventory Data**

*B. Kuczenski, University of California, Santa Barbara; P. Arbuckle, E. Kahn, USDA*

The current paradigm for life cycle inventory data management, typified by large, centrally maintained databases that are monolithic and infrequently updated, has struggled to provide the granularity and representativeness required for many studies. At the same time, there is no easy way for authors of different LCA studies to collaborate or share their work with one another. While the topic of data interchange and interoperability receives a lot of attention, comparatively little work has focused on workflows and best practices for making data and models available for use. We address this gap by considering the different kinds of information that are relevant to preparing and sharing LCA studies, and the roles of different agents in supporting the task of building a life cycle model. LCI data are often organized in terms of unit processes and flows, because these formative concepts are ubiquitous in the standards and the literature. However, we find they are not well suited for organizing practitioner knowledge. We find that life cycle data contains three distinct modalities, each of which requires a different approach for organizing and sharing. By volume, the vast majority of information in an LCI database is qualitative, also known as metadata, which includes scope, system boundary, representativeness and methodological characteristics. The quantitative mode, by contrast, includes limited, strictly numeric data: the amount of a given flow that is associated with the activity of a given process. We identify a third, semi-quantitative mode that is often neglected in LCI data discussions, namely the adjacency of processes, or which processes are partners in an exchange. For all three modalities, the exchange, and not the unit process, is the most "atomic" unit for storing and sharing knowledge. Beyond the exchange, the product system (and not the unit process) is more representative of how knowledge about systems is gathered and organized. From this perspective, unit processes are aggregations of product systems -- derived rather than primary datasets. We will discuss the differing roles and interests of practitioners, study commissioners, academic researchers, data providers, public agencies, and non-governmental organizations in creating and curating these different kinds of information.

**WP129 LCA and AA: An Integrated Screening Approach**

*H. Plugge, Verisk 3E; E. Griffing, Environmental Clarity; M. Overcash, Wichita State University / Industrial, Systems, and Manufacturing Engineering Department*

In the last few years there has been a trend from detailed assessments at the end of a product design cycle, to screening of risks in an earlier phase, the so-called Test Early, Fail Early paradigm. Various risk screening methodologies have been initiated at the hazard and risk level. A screening approach to LCA is more of a novelty, especially when integrated with health and environmental hazard and risk screening. Alternatives Assessment (AA) especially cries out for a screening approach. The original NAS AA report about 4 years ago described an integrated approach for performing AA's which included LCA in the decision scheme. Previous presentations by the authors have suggested incorporation of an integrated screening approach early in the AA scheme, in order to allow preliminary screening of alternatives, without engaging in multimillion dollar full-blown assessments. A rapid preliminary decision can be reached albeit commonly on an incomplete dataset. The initial tenet of AA is deceivingly simple: maintain functionality. Substitution will never succeed if the alternative does not perform at least equal to the original chemical. Screening health and environmental effects can be calculated using 3E's Green Score methodology. Environmental Clarity 's Life Cycle Inventory (LCI) methodology provides a rapid screening LCA estimate based on process engineering based LCI data. NRE (Natural Resource Energy) and GWP (Global Warming Potential) metrics are used on a cradle to gate (CTG) scope. Results are then presented in a decision matrix allowing (de)selection of alternatives. A case study will be presented based on alternatives and analogues to C3 and C4 alcohols. Contrary to common assumptions C4 alcohols are not at all similar with regard to their physicochemical properties, slightly similar with regard to health effects and more similar with regard to LCA impacts and environmental effects. Screening data for the series ethanol through 1-pentanol will be presented and decision pairs selected and screening estimates provided for all. None of the butanols are equivalent/provide a good substitute to tert-butanol, although isopropanol does, based on functionality.

**WP130 LCA Data Management – Current Status and Future Directions**

*B.W. Vigon, Breveja Environmental Consulting LLC*

Since the publication of the Global Guidance Principles for LCA Databases in 2011, a great deal has been done to develop and implement in a practical way, the recommendations of that workshop. This presentation will summarize those efforts and provide an integrative perspective on how the various activities complement one another. In particular the projects of the Life Cycle Initiative, both the previous phases and the current one, the UN Environment REAL Project, and the GLAD Network, will be covered. The objective of this look-back will be to both highlight earlier identified needs in the data arena and spotlight gaps that still should be addressed going forward. Efforts to date have included development and roadtesting of data review procedures and criteria. These criteria have been or are being incorporated into the meta-data fields available when users log into the GLAD portal. In a parallel vein a basis for assessing database conformance to recommended management practices first listed in the Global Guidance Principles, have been elaborated and tested on multiple databases in developed and developing countries. Both of these advancements have been supported by the Life Cycle Initiative and the UN Environment LCA Databases Helpdesk.

**WP131 LCA in organizations: It can tackle climate change?**

*W.H. Motta, IBICT / Information Science Post-Graduation Program*

Climate change is increasingly recognised as a major challenge, among other problems arising from the experienced ecological crisis. It is widely accepted that the greenhouse gas emissions caused by humans are having a negative impact on the environment, causing serious implications for the ecosystem and consequently for human life. Contributed in a sweeping way in the alteration of the carbon cycle and other cycles of matter and energy in the terrestrial system, leading to the term "Anthropocene" for the present time. Particularly after the commitment that 195 nations made through the Paris agreement (COP 21), the issue became even more widespread and has positioned itself at the top of the political environmental agenda and as one of the main environmental issues addressed by society in general. Due to this reality, the growing awareness of the need for environmental sustainability in personal life and at work has increasingly brought "sustainable" practices to the agenda of organizations. Environmental constraints are increasingly being imposed on organizations and require new levels of operational compliance, thus promoting the need to apply and use new methodologies and tools. Among these methodologies can be pointed out Life Cycle Assessment – LCA, that has been increasingly accepted as a way to assess the environmental impacts associated with all stages of a product's life. It encompasses the entire production chain: the extraction of natural resources, transport, production process, consumption and disposal of products (waste). Taking into account an LCA study being performed considering only the impact category of greenhouse gas emissions, it can for example consider from direct use of fuels to indirect impacts such as employee travel or emissions from other organisations up and down the supply chain. In this context, could LCA in organizations contribute to tackle climate change? In what way has LCA been used by organizations? The present study aims to shed light to this discussion through a literature review analysing the use of LCA by organizations and its potential facing climate change challenges. The evolution of the use of LCA by organizations promises fruitful results in this regard.

**WP132 Life cycle assessment of aquaponics and agriculture informed by literature review**

*F. Wu, University of Wisconsin, Madison / Chemical, Biological and Environmental Engineering; A. Hicks, S. Jacobsen, University of Wisconsin, Madison / Department of Civil and Environmental Engineering*

As the global population continues to grow and resources continue to dwindle, there is an abundant need for prolific and sustainable food systems. Recently, the interest in aquaponics has grown. The number of commercial facilities in the US has more than doubled from 71 to 145 from 2013 to 2015. Aquaponics, a closed loop production system of food and produce products, is one emerging method of food production which may have much lower environmental impacts than conventional food systems, while providing an environmentally sustainable protein source. Fish excrete ammonia which is broken down by bacteria into nitrite. The plants utilize the nitrite and act as filters by removing the nitrite from the recirculating water. By employing the symbiotic relationship between the fish and the plants, aquaponics can reduce the amount of inputs needed and lessen the impact on the environment compared to traditional farming. Life cycle assessment (LCA) is a widely accepted and useful tool that assesses the environmental effects of products and services. Thus, LCA is a holistic approach that quantifies the direct and indirect environmental impacts and can help identify the most sustainable and least sustainable aspects of a product or service. This poster is aiming to introduce and compare the current LCAs that have been performed on aquaponic systems at relative environmental categories to date. Based on current information, most systems use tilapia, a warm water fish, and a variety of vegetable are grown the most popular being herbs, lefty greens, and tomatoes. In addition, although aquaponics offers a promising sustainable food system solution, the profitability of the system can vary depending on location, laws, species of vegetables and fish grown, and markets the products are sold in. This poster review also looks at the techno-economic assessments that have been performed on aquaponic systems.

**WP133 Environmental and economic performance evaluation of decentralized wastewater treatment plants in India using life cycle approach**

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Life cycle assessment (LCA) was used to evaluate the environmental impacts associated with wastewater treatment plants (WWTPs). Six most commonly used wastewater treatment technologies in India, namely; Sequencing batch reactor (SBR), Membrane bioreactor (MBR), Moving bed biofilm reactor (MBBR), Soil biotechnology (SBT), Aerated lagoons (AL) and Activated sludge process (ASP) have been evaluated. CML Baseline 2000 method was used for assessing life cycle impacts considering eleven potential impact categories. The scope of the study considered operation and maintenance phase, sludge treatment and landfilling, and the transportation of sludge to landfill. Moreover, an economic evaluation was also addressed using life cycle cost (LCC) approach employing present worth method. It is evident from LCA that emissions associated with electricity production for operating the WWTPs, emissions into water bodies from the treated effluent and hazardous heavy metals emissions into effluent and waste sludge have been identified as the main contributors to the overall environmental impact. Marine aquatic ecotoxicity potential was found to be the most significant impact category in the WWTPs under consideration. Among the wastewater treatment technologies, SBT obtained the lowest environmental impact in all the evaluated impact categories, except for eutrophication potential. While, the AL system presented the worst results due to the high electricity and chemicals consumption. On the other hand, the present worth of SBT was estimated to be Rs. 40 million/MLD which is highest as compared to other technologies. This high cost is due to land requirement and high density polyethylene liner cost. MBR is second highest (Rs. 24.7 million/MLD), which is mainly contributed by civil, electro-mechanical and membrane cost. The results of LCA and LCC provide specific insights about the factors which play a major role during the life cycle of wastewater treatment technology and its associated impacts. A comprehensive LCA and LCC have been documented in the present study which can help decision makers to take well informed decisions simultaneously addressing sustainability.

## Assessing Contaminant Effects on Early Life Stages of Marine Organisms

**WP134 Physiological consequences of environmental contamination in an elasmobranch with matrotrophic histotrophy, the Round Stingray**

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Despite widespread evidence of negative reproductive effects in most other vertebrate taxa from organic contaminant exposure, similar studies in elasmobranchs are limited. We examined a range of physiological biomarkers in two populations of Round Stingray (*Urobatis halleri*) that differed primarily in PCB exposure in southern California (USA). Females, and their embryos, were sampled each month of pregnancy at both sites, while adult males were matched with a 40-day subset of females to investigate the effect of sex and its interaction with PCB exposure and development. Exposure was hypothesized to have negative outcomes for PCB-exposed stingrays compared to the reference population. We found a variety of impacts, ranging from reproduction to osmoregulation. Female fecundity was not affected by PCB exposure; however, exposure exacerbated maternal tissue mass, and quality, loss during pregnancy. Embryos from contaminant exposed sites appeared to not use maternal resources as efficiently as their reference counterparts. Sex-related differences in embryo mass differed between sites, with males being relatively heavier than females in the reference site but not the expose site, suggesting contaminant effects on males begin *in utero*. These contaminant sex-related effects extended into adulthood, as relative liver mass and energy content were lower in comparably-sized adult males than females from the exposed site, whereas fewer differences were found between adults at the reference site. Effects found both *in utero* and in adulthood suggest that contaminants have a significant, and potentially life-long, impact on Round Stingray homeostasis. This has implications for other species with greater contaminant burdens. Contaminant exposure, and its interactive effects with sex and age, should be included as part of effective elasmobranch management.

**WP135 Accumulation, elimination and neuro-oxidative damage under lanthanum exposure in glass eels (*Anguilla anguilla*)**

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Rare earth elements (REEs) comprise elements from lanthanum to lutetium that together with yttrium and scandium are emergent contaminants of critical importance for numerous groundbreaking environmental technologies. Transfer to aquatic ecosystems is expected to increase, however, little information is known about their potential impacts in marine biota. Considering the endangered conservation status of the European eel (*Anguilla anguilla*) and the vulnerability of early fish life stages to contaminants, we exposed glass eels, through water, to an environmentally relevant concentration (120 ng.L-1) of lanthanum (La) for 7 days (plus 7 days of depuration). The aim was to study the accumulation and elimination of La in eel's body and subsequent quantification of acetylcholinesterase (AchE), lipid peroxidation and antioxidant enzymatic machinery. Accumulation peaked after 72h-exposure to La, decreasing afterwards, even in continuous exposure. Accumulation was higher in the viscera, followed by the skinless body and ultimately in the head, possibly as a protective mechanism to cope La neurotoxicity. A significant increase in AChE activity was observed in La-exposed glass eels, suggesting that $La^{3+}$ may inhibit the binding of acetylcholine. A depression in lipid peroxidation was registered under La exposure, possibly indicating that $La^{3+}$ may play physiological activities and functions as a free radical scavenger. Catalase activity was significantly inhibited in La-exposed glass eels after 72h, indicating that the availability of La may induce a physiological impairment. The quantification of Glutathione S-Transferase activity revealed no differences between control and La-exposed organisms. Further investigation is needed towards understanding the biological effects of REEs.

**WP136 Assessment of cardiac toxicities in embryos of marine medaka exposed to oxygenated PAHs spiked into artificial sediment**

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Many kinds of chemicals have been residues in sediments of coastal area, especially closed to the big cities and industrial zone. Some of them are persistent such as polycyclic aromatic hydrocarbons (PAHs), and possibly affect especially in the benthic animals. Oxygenated PAHs (oxyPAHs) have one or more oxygen molecules connected by a double bond to the benzene rings in PAHs. They are discharged directly into the environment with the exhaust gas from diesel engine automobiles and flue gases from various combustion process. After transferring into the water columns, oxyPAHs absorb on the micro-particles and can settle on sediments in the coastal area. Previously, we reported the cardiac malformation caused in freshwater medaka embryos exposed to oxyPAHs such as 7,12-benz(a) anthracenequinone (BAQ) and 9,10-phenanethrenequinone (PHQ). From these results, oxyPAHs possibly cause the cardiac toxicities to fish embryos. If we can find the diagnostics of cardiac toxicities in the early embryos of fish, we consider that the potencies of cardiac toxicities even in larvae can be predicted. In the present study, we tried to assessment the cardiac toxicities in the embryos of marine medaka, Java medaka (*Oryzias javanicus*), exposed to oxygenated or parent PAHs spiked into the artificial sediments. The examined compounds were BAQ, PHQ, 1,4-naphthoquinone (NAQ) as oxyPAHs, and pyrene as parent PAH. Those were spiked into the artificial sediments made with reference to TG218 in OECD test guideline. Ten embryos were embedded in half of them in sediment, which was laid at a glass petri dish, and were developed on the sediment for 12 days post-fertilization (dpf). In the period, the rearing water was not added to the dishes, and sediment contained slight amount of pore water (artificial sea water). The embryos at 5 or 12-dpf were shot video under a microscope. Then, only the cardiac part in the video were left, and others were cropped. The temporal variations of areas on the heart were crimped. We regarded the crimp as a similar to electrocardiogram, and determined arrhythmic heartbeat after measuring heartbeat. In this method, we could clearly observe the cardiac abnormalities for heartbeat and arrhythmic heartbeat in embryos at 12-dpf compared with them at 5-dpf. Especially, NAQ tended to be increased the frequencies of arrhythmic heartbeat in embryos even under lower concentrations (0.33 mg/kg), NAQ can sensitively cause cardiac toxicities.

**WP137 Potential Population-level Impacts of Oil Spills on Puget Sound Pacific Herring Stocks**

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Localized oil spills that contaminate nearshore spawning areas pose a threat to developing Pacific herring embryos. Fish embryos are particularly susceptible to the developmental toxicity of oil-derived PAHs, which can be acutely lethal or, at lower exposure doses, lead to permanent changes in heart structure, craniofacial morphology, and metabolic processes that cause delayed mortality, or more subtle delayed impacts that may be associated with premature subadult mortality. A variety of localized oil spill scenarios simulating direct and delayed mortality to young of the year were used to examine the potential response of Puget Sound stocks. The healthy stocks remaining in Puget Sound could withstand short term impacts, but the growing number of depressed and unhealthy stocks are vulnerable to an increased risk of localized extinction. Model output predicted that for short term, low level impacts the stock abundance did not exceed the natural variability observed in the population demographic data. This indicates a limited ability to observe in the field any predictions made by the model. High mortality in a single year or impacts across multiple year classes may cause stock abundance changes that exceed natural variability. Despite this, the model does indicate the magnitude of impact on the intrinsic growth rate that could reduce productivity of affected stocks. Additional toxic endpoints and effects thresholds are currently being investigated, including sublethal impacts on cardiac function, immune function, and lipid metabolism that may all lead to delayed mortality. Characterizing these adverse outcome pathways may alter predicted impacts of oil spills on herring stocks by informing, systematic post-spill field assessments measuring exposure concentrations and assessing the proportions of herring embryos, larvae, or juveniles showing specific phenotypes (e.g., cardiac edema) or gene regulation patterns indicative of changes in individual fitness via swimming ability, immune function, or lipid metabolism. These data may be useful for any injury assessment for Pacific herring.

**WP138 Crude oil cardiotoxicity and growth throughout development in Pacific herring (*Clupea pallasi*)**

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Several years after the 1989 Exxon Valdez oil spill, the Pacific herring (*Clupea* pallasi) population of the Prince William Sound crashed and never recovered. Despite finding that larval herring are extremely sensitive to low concentrations of polycyclic aromatic hydrocarbons (PAHs), the connection between oil spill events and delayed population-level effects has yet to be established. The developing heart is the primary target of crude oil toxicity and therefore the most likely starting point for the cascade of events leading to reduced recruitment at the population level. By tracking cardiac morphology and growth between larval hatch and metamorphosis to juveniles, this study was intended to evaluate relationships between embryonic cardiotoxicity and delayed population impacts of oil spill events. Herring embryos were exposed to six concentrations of oil ranging from 0 to 5.1 ppb ∑PAHs from 24 hours post fertilization to 10 days and subsequently reared in clean sea water. Oil concentrations were maintained by a dispersion generator which injected microdroplets of Alaskan North Slope crude oil into the exposure system. Larval length, cardiac edema, and ventricle and atrium area and shape were assessed weekly by video microscopy through metamorphosis, approximately 80 days post hatch. Preliminary results indicated that the cardiac morphology of larval herring was more sensitive to oil than the analytical methods for detecting waterborne oil. At near-detection limit concentrations, we observed cardiac edema, reduced ventricular area, enlarged atrium, and altered ventricular shape. This change in shape can reduce cardiac output and consequently aerobic capacity, which could impact survival as fish reach adulthood. This work is part of a larger collaboration intended to evaluate the effects of larval cardiovascular morphology, lipid composition, and gene expression on juvenile bioenergetics and susceptibility to infectious and parasitic diseases.

**WP139 Development of a Novel Acute Toxicity Method for a Marine Copepod: Sensitivity to Copper, Phenanthrene and Ammonia**

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There are limited acute (48 hour) toxicity test methods for marine species that are considered zooplankton for their entire life cycle. Examples of current standardized marine zooplankton methods include mussel, bivalve and echinoderm development tests that focus on planktonic life forms that consist of only a very short duration of the organism's life cycle or chronic life cycle copepod toxicity tests that involve epibenthic species. A currently available ISO method lists an acute, 24 hour test using a marine planktonic species of copepod that is not indigenous to North America. The objectives of the present study were to (1) develop a novel 48 hour acute toxicity test method using a marine copepod species indigenous to North America that is planktonic for its entire life cycle; (2) make modifications to the test method applicable to sediment elutriate toxicity testing; and (3) determine the sensitivity of this copepod to copper, phenanthrene and ammonia relative to other commonly used marine toxicity test species. The calanoid copepod *Pseudodiaptomus pelagicus* was selected as the test species and was exposed to each of the three model toxicants in five separate 48 hour toxicity tests. The average lethal median concentration (LC50) values for copper, phenanthrene and un-ionized ammonia were 33 ± 13 µg/L, 161 ± 51 µg/L and 1.08 ± 0.30 mg/L. Toxicological endpoints were generated by summarizing data based upon dissolved chemical stability in the water column, with arithmetic means used for copper and un-ionized ammonia and geometric means for the less stable dissolved concentrations of phenanthrene. These LC50 values placed *P. pelagicus* on the more sensitive range of copper and phenanthrene species sensitivity distributions. The copepod was less sensitive to un-ionized ammonia than mussel and urchin development toxicity tests. The results suggest *P. pelagicus* is relatively sensitive to typical contaminants of concern released to the water from sediment elutriates but less sensitive to naturally occurring ammonia levels released from sediments. No effect levels calculated as USEPA benchmark dose low values were determined to provide a frame of reference for when effects due to these chemicals are not expected to occur. Additional research developing this method includes an inter-laboratory comparison.

## Tackling Urban Background: Approaches, Case Studies and Policy Implications

### WP140 Background and Bioaccessible Concentrations of PAHs in Florida Urban Soils

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Soil cleanup standards for contaminated soils can be based on either soil background concentrations or risk-based values. Mounting evidence suggests that understanding contaminant bioavailability in soils is necessary for accurate assessment of contaminant exposure to humans via oral ingestion pathway. Polycyclic aromatic hydrocarbons (PAHs) are a group of contaminants come from biogenic, petrogenic and pyrogenic sources in the environment. The objective of this study was to determine the total and bioaccessible concentrations and distributions of PAHs in Orlando and Tampa urban soils. The results showed the soils were dominated by high molecular weight PAHs in both cities. Benzo(a)pyrene equivalent (BaP-EQ) concentrations in 60-63% samples were higher than residential Florida soil cleanup target level (FSCTL) and 20-25% samples were higher than industrial/commercial FSCTL in Orlando and Tampa. Orlando soils had relatively higher bioaccessible PAHs and BaP-EQ concentrations than Tampa soils, and both cities had similar PAHs bioaccessibility which ranged from 0 to 93%. The bioaccessible BaP-EQ concentrations in both cities were under industrial/commercial FSCTL. High molecular weight PAHs had relative lower bioaccessibility than low molecular weight PAHs due to the hydrophobic characters. Based on molecular diagnostic ratios and PMF model, major sources of both total and bioaccessible PAHs in both cities were similar, mainly coming from pyrogenic, traffic emissions, and biomass and coal combustion. Based on ArcGIS maps, total PAHs concentrations in central business district and areas near high traffic roads were significantly higher than the other areas in both cities.

### WP141 Characterizing Urban Background Levels for Contaminated Site Cleanup

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Identifying the source of soil contaminants is vital to decision-making during an environmental cleanup. Soil in long-established cities has accumulated decades of low level concentrations of metals and other chemicals like polynuclear aromatic hydrocarbons (PAHs) caused by urban activity. The combination of natural and anthropogenic background levels of chemicals is known as urban background contamination. These metals and chemicals can intermingle with higher concentrations of chemicals from spills and industrial waste creating challenges in understanding how industrial site contamination is contributing to overall soil concentration levels and in establishing protective and achievable clean-up goals. EPA and state officials collaborated on a multi-city sampling study created to assist hazardous waste site managers differentiate between site-related contamination and contamination from nearby non-point sources (urban background contaminants). This study goal was to design a data acquisition process to characterize levels of contaminants in a city that derive from natural and urban infrastructure sources not related to chemical spills and industrial waste and to develop a project database in which soil concentration data and sampling location metadata are compiled for all sampled cities. EPA worked in cooperation with representatives from the Southeast Region States (Florida, Georgia, Kentucky, Mississippi, North Carolina, South Carolina, and Tennessee) to develop a unified plan for the selection of sampling locations and implementation of a consistent sampling approach for application across all States. The finalized plan was applied at eight cities to evaluate implementation consistency and provide training to participating State personnel then used in a case study to aid in establishing background concentrations for a Superfund site.

### WP142 The Role of Urban Background in Site Investigations: A Spectrum of Ramifications

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Many contaminated sites being investigated under federal and state regulatory programs are located in urban centers with their own contamination problems from decades of historical mixed industrial and commercial land use. This presents unique challenges for site investigations in urban centers, as opposed to less urban areas where the levels of contaminants in the ambient environment can be clearly distinguished from the site contaminant sources. This presentation will discuss some recent studies characterizing urban background and explore case studies that demonstrate the spectrum of ramifications of urban background on the remedial action process. The focus will be on priority pollutant polycyclic aromatic hydrocarbons (PAHs) and metals, which have been the focus of the author's own evaluations, and which have been the recent focus of urban investigations by Region 4 of the U.S. Environmental Protection Agency. Some of the ramifications to be demonstrated by the case studies include 1) the effects of urban background on site investigation design, 2) the effects of background on risk assessments performed to address potential human health and ecological risks, and 3) the effects of urban background on the ultimate remedial footprint selected for remedial action. Lastly, a path forward will be proposed for future work that needs to be completed to better address the ramifications of urban background in regulatory policy-making.

## Canadian Oil Sands: Advancing Science in Chemical and Toxicological Characterization, Reclamation and Monitoring

### WP143 A model of contaminant removal from oil sands process-affected water in the Kearl treatment wetland

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Large volumes of Oil Sands Process-affected Water (OSPW) are produced by the Canadian Oil Sands during bitumen extraction. The safe management of this OSPW poses a challenge to industry operations since no discharge of this wastewater is permitted at this time. OSPW is therefore stored in large tailings ponds while industry continues to search and develop feasible and effective solutions to manage this wastewater. One potential solution is to remediate OSPW through passive treatment with treatment wetlands. These systems have been successful at remediating municipal and domestic wastewater, animal wastewater, mine water, industrial wastewater, urban storm water, field runoff, and leachate. The Kearl Treatment Wetland (KTW) is a 1 ha surface-flow treatment wetland containing 3 deep pool and 3 shallow areas, and located on Imperial's Kearl Oil Sands site in northern Alberta, Canada. In this study, we investigate the removal of polycyclic aromatic hydrocarbons (PAHs) using polyethylene samplers (PES) that were deployed in the inlet and outlet of the KTW for two consecutive deployments (July 21 – August 29, 2017, and August 29 – September 29, 2017). The performance of the treatment wetland was evaluated using these estimates of the freely dissolved PAH concentrations at the inlet and the outlet of the KTW. These data were also used test and calibrate a previously developed and parameterized contaminant-fate treatment wetland model that estimates contaminant removal via various biogeochemical processes (volatilization, transpiration, microbial degradation, plant-enzyme biotransformation, sorption to rooting media, burial). There was a consistent decline in analyte concentrations throughout the KTW during both deployments. There was a greater average change ($p < 0.05$) in concentration of contaminants measured in deployment 1 (71.7%, SD 24.2%; n = 20) compared to deployment 2 (39.1%, SD 32.1%; n = 27) resulting from changes to environmental conditions (i.e. precipitation, evapotranspiration, temperature). Model calibration resulted in an overall model bias of 1.02 (95% CI 0.75 – 1.39) suggesting a strong fit of the model estimates and the empirical data. The calibrated model showed that a log $K_{OW}$ (octanol-water partition coefficient) of 3.5 – 5.5 and a log $K_{OA}$ (octanol-air partition coefficient) less than 5.0 result in the highest removal efficiencies through the KTW.

**WP145 Assessment of liver tumor incidence in white sucker collected to determine baseline fish health in the Alberta Oil Sands area**

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In 1987, the International Joint Commission identified 43 Areas of Concern (AOC) in the US/Canadian Great Lakes. To qualify as an AOC, the area contained one or more beneficial use impairment (BUI). One of the BUIs identified at a number of locations was fish tumors or other deformities. It was defined as when the inciidence rates of liver tumors or other deformities exceed rates at unimpacted control sites or when survey data confirms the presence of neoplastic or preneoplastic liver tumors in bullheads or suckers. As part of the Joint Canada-Alberta Oil Sands Monitoring Program (JOSMP), fish health within the Athabasca watershed is being evaluated using methods developed for the Canadian Environmental Effects Monitoring programs. On the Athabasca River mainstem, white sucker have been selected as a large bodied sentinel species and 3 years of baseline fish health data has been collected at five sites. During these collections, liver samples were preserved for liver tumor assessment similar to those for the Great Lakes AOC. With three years of sampling 20 males and 20 females for fish health from each site, a sample size large enough to evaluate liver tumor incidence and potential increases due to exposure to natural oil sands compounds from the outcrops of the deposit or potential increase incidence due to increased development could be assessed. As part of the increased geographical extent of the JOSMP program, white sucker were also collected from Lake Athabasca during the fall of 2015 and sampled for liver tumor assessment. Samples were processed in the Environment and Climate Change histological laboratory and final assessment of the slides by the Animal Health Center of the British Columbia Ministry of Agriculture. Final results are still being collected and assessed, but liver tumor incidence will be compared between fish collected at reference sites upstream outside of the oil sands deposit, within the deposit upstream of development, in the deposit downstream of development, from Lake Athabasca further downstream and to reference fish collected within the Great Lakes. PAH data from muscle and liver from a limited number of these fish from these sites will also be presented along with hepatic mixed function oxygenase activity as an indicator of exposure of these fish to PAH related compounds.

**WP146 Development of Aquatic Environment Monitoring Triggers for the Lower Athabasca River**

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Oil sands companies currently do not release process affected water to receiving environments, but there is a need and desire to do so in the future. Release of depressurization water is currently limited, but has shown no measurable changes in the receiving environment where this practice has been ongoing since 2008. There are concerns about the potential for effects of the discharge of process waters to the Athabasca River. Environmental Effects Monitoring (EEM) provides a mechanism to test predictions about the potential effects of effluent releases. EEM programs are currently in place for metal mining and pulp and paper sectors in Canada, and have demonstrable efficacy at quantifying effects and identifying potential effluent-related causes of effects. Fish populations have been extensively monitored in the mainstem of the Athabasca River under the historical Regional Aquatics Monitoring Program (RAMP) and the Joint Oil Sands Monitoring Program (JOSMP). Those data can be used as a baseline to judge the potential effects of future process water releases from oil sands operations. The results herein originated from the compilation of fish population (Trout Perch) data that were collected under RAMP and JOSMP from 2009 through 2015. Normal ranges of various measures of fish population performance were modeled in relation to river discharge and air temperature. The resulting models were statistically significant and ecologically meaningful, and thus, provide predictions or forecasts of future conditions for monitoring the potential effects of point-source discharges to the Athabasca River.

**WP147 In vitro assessment of pH-dependent uptake and toxicity of ionizable organic chemicals in fish**

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Uptake and effects of ionizable organic chemicals in fish can significantly differ as a function of ambient pH. These differences are driven by the rate of passive diffusion of the uncharged species across the gill epithelium, which is considerably greater than that of charged species. Consequently, the flux of chemicals will peak at different pH values depending on their acid dissociation constants. Here, we propose a rapid in vitro screening method to assess the pH-dependent uptake of ionizable organic chemicals, specifically weak acids, at the fish gill. To this end, the permanent rainbow trout gill cell line RTgill-W1 was grown in transwell tissue culture inserts for two to three weeks, and allowed to establish tight monolayers as characterized by stable transepithelial electrical resistance (TEER). After acclimatization to the reduced complexity exposure medium L15/ex at pH 6.0, 7.4, or 8.5, the permeation of chemicals from apical to basal transwell chambers was determined during a 24-h exposure period by means of liquid chromatography with high resolution mass spectrometry (LC-HRMS). The neutral red retention assay was conducted prior to exposures to exclude interference of cytotoxicity with the measurements. The assay was then used to investigate individual model chemicals (chlorophenols, carboxylic acids) and a technical mixture (nonylphenol) that had been previously shown to cause pH-dependent toxicity. To explore the applicability domain of the assay, we chose to investigate the pH-dependent permeation of chemicals present in acidic, neutral, and basic fractions, as well as reconstituted total extracts of oil sands process-affected water (OSPW). The acute toxicity of OSPWs has been shown to be mostly associated with the acidic fraction, specifically naphthenic acids. We observed a substantial pH-dependency of the cytotoxicity and permeability of weak acids, as well as the acidic fraction and reconstituted total extract of OSPW. We conclude that our in vitro assay can be used to screen for pH-dependent uptake and toxicity of ionizable organic chemicals in fish. It is intended to validate the test for application to prediction of pH-dependent uptake and effects in vivo. Thus, it may be a highly valuable tool for in vitro-in vivo extrapolation, and prioritization of chemicals in non-target chemical screenings.

**WP148 RNA-sequencing to identify effects of oil sands process-affected water in chronically exposed *Daphnia magna***

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Extensive research has been conducted on the acute toxicity of bitumen-influenced waters and the organic mixtures therein, with assessment of multiple endpoints from organisms of varying taxa. However, there has been a paucity of research focused on sub-lethal or chronic endpoints. Assessment of toxicity endpoints other than acute lethality will provide data of great importance to initiatives intent on monitoring and reclamation within the oil sands region. In the present study, next-generation RNA-sequencing was used to identify biological pathways impacted by the chronic exposure of the model organism *Daphnia magna* to different oil sands process-affected water (OSPW) extracts. One groundwater sample with a solely natural bitumen influence, and a second groundwater sample with a combination of influences from natural bitumen and OSPW, were collected from within the McMurray oil sand formation and fractionated utilizing differences in polarity in an effects-directed analysis approach. Lethality was not observed in 48-h acute assays with *D. magna* assessing the toxicity of soluble organic fractions from different bitumen-influenced groundwaters at 100% whole water equivalents (WWE). Chronic 21-d exposures were conducted for all primary fractions at 25% WWE to ensure survival throughout the duration of the test and to avoid unspecific stress-related gene transcription profiles. Total RNA was extracted from daphnids at the end of exposure and RNA-sequencing of 75 bp paired-end reads performed using an Illumina MiSeq instrument. *De novo* transcriptome assembly and statistical analysis were done using CLC Genomics Workbench pipeline. Quantitative PCR analyses will be conducted to validate the genomic data and cellular markers of interest will be identified, analyzed and compared between fractions. The use of this high throughput technology will help identify potential pathways affected by exposure to OSPW and help align future effect research in aquatic organisms.

**WP149 The impacts of diluted bitumen oil spills on freshwater phytoplankton communities**

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The impacts of unconventional oil spills in fresh waters are currently poorly understood at the ecosystem-level. New oil pipelines have been proposed across North America to bring diluted bitumen (dilbit), a heavy crude oil diluted with light gas condensates to facilitate transport through pipelines, from the Canadian oil sands region to refineries and other markets. These pipelines span a large geographic area, and in many cases, transverse or come into close proximity of inland water bodies. However, it is unclear how an accidental dilbit spill will affect these freshwater ecosystems. As part of the collaborative BOREAL (Boreal lake Oil Release Experiment by Additions to Limnocorrals) research project, we seek to give a comprehensive picture of the environmental fate of dilbit, its impacts on ecosystem structure and function, and its toxicity to higher trophic levels within a boreal lake ecosystem. At the IISD-Experimental Lake Area in north-western Ontario, Canada, we installed a series of nine limnocorrals (10-m in diameter and 2-m deep) in the littoral zone of a remote lake to test how dilbit affects freshwater ecosystems across a range of environmentally realistic dilbit spill volumes (ranging from 1:100,000 to 1:1,000 oil-to-water ratios). In this subproject, we examined how these experimental dilbit spills affected the structure and function of the phytoplankton community, which constituent an important component of the ecosystem at the base of the aquatic food web. To assess whether dilbit reduced algal biomass, we tracked chlorophyll *a* and flow cytometry for over 10 weeks post-spill. At the same time, we tracked changes in phytoplankton community structure by assessing samples using light microscopy (to species). Finally, we assessed primary production through dissolved oxygen concentrations and Pulse-Amplitude-Modulation to test for impaired photosynthesis via photosystem II. This research will advance our scientific understanding of the effects of unconventional oil in fresh waters and we anticipate our findings will be used by policy makers to develop safe dilbit transportation policies and for consideration in environmental monitoring and remediation strategies.

**WP150 Effects of diluted bitumen on wild small-bodied fish: A freshwater mesocosm study at IISD-Experimental Lakes Area, Northwestern Ontario**

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A collaborative field study examining the fate, behaviour, and potential toxicological effects of diluted bitumen (dilbit) in fresh water began in 2018 in a boreal lake at the IISD-Experimental Lakes Area (Ontario, Canada). The Boreal lake Oil Release Experiment by Additions to Limnocorrals (BOREAL) project examines model spills of dilbit in seven littoral mesocosms (10m diameter, ~157,000 L volumes) to achieve dilutions ranging from 1:100,000 to 1:1,000 (dilbit:water). Two additional mesocosms, not treated with dilbit, were included as controls. Here we report the effects of chronic exposure (>21 days) on free-swimming, wild finescale dace (*Phoxinus neogaeus*). Exposure was determined by measuring polycyclic aromatic hydrocarbons (PAHs) in tissues and metabolites in the bile. Adult male and female finescale dace were exposed in order to assess reproductive health, while juveniles were included to assess growth. Both life stages were used to assess physiological and molecular responses to dilbit. Assessed metrics of reproductive health and metabolism include calculation of male and female gonadal somatic indices, egg diameter, histological development of gonads in both sexes, and condition factor. Gill and liver histology were examined as well as molecular markers (CYP1A, GST, soc1). Oxidative stress was determined by measuring isoprostane concentrations in tissues. The BOREAL study provides toxicological effects data from the first controlled in situ ecosystem level exposure of dilbit in freshwater.

**WP151 Sublethal Cardiac and Metabolic Effects of Crude Oil in Fathead Minnow (*Pimephales promelas*)**

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Transport of petroleum products in Canada is becoming an increasing public and political concern, in part due to the potential for unintended impacts on aquatic ecosystems. These concerns have necessitated further study, as the current understanding of petroleum toxicity is limited in a number of ways. Specifically, much of the existing research is focused on characterizing early life-stage developmental toxicity, however limited evidence suggests that there may also be sublethal effects on cardiac and metabolic endpoints in adult fish exposed to crude oil. Such effects can potentially impact fish populations by adversely affecting predator avoidance, reproductive success, and overall fitness. Additionally, much of the existing research has focused on specific fractions of petroleum products as opposed to characterizing toxicity of whole compounds. Petroleum compounds are complex mixtures of polycyclic aromatic hydrocarbons, naphthenic acids, and other potential toxicants; so it is crucial to examine the effects of these compounds as they would occur if released to the environment. The aim of this research is to address some of these gaps identified in the literature by examining sublethal cardiac and metabolic effects in individuals of a native fish species (Pimephales promelas) that were sub-acutely exposed to environmentally relevant concentrations of a whole-compound Canadian crude oil. A water accommodated fraction (WAF) of crude oil was prepared by mechanical dispersion and used to dose receiving waters, simulating exposure resulting from an aquatic spill. Fish were then exposed to concentrations of either 0% (Control) 0.3%, 3%, or 30% WAF for 7 days in a static 48 hr renewal protocol. Following the exposure, cardiac structure and function was assessed using ultrahigh-resolution B-mode and pulse-wave Doppler ultrasound. A second parallel set of exposures was carried out under the same parameters in order to assess fish for swim performance and standard metabolic rate (SMR). Swim performance was assessed using swim tunnel respirometry, whereas SMR was assessed using intermittent flow respirometry. The results of this research will add to our understanding of sublethal toxicities of complex petroleum mixtures to adult fishes. Future work will focus on characterization of diluted bitumen toxicity, as well as comparison of the results between the two studies.

**WP152 Impacts of Petroleum Toxicity on Marine Mammals, Reptiles, and Fish: A Review**

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With the continuing potential for increased shipment of both conventional crude oil and unconventional petroleum like diluted bitumen out of the port of Vancouver, there is a need to assess the toxicological consequences of chronic or catastrophic petroleum spillage on the marine wildlife of Canada's Pacific coast. Currently there are only a handful of papers in the peer reviewed literature on the effects of diluted bitumen on marine wildlife. Additionally, there is a need for a current and comprehensive literature review of petroleum toxicity on marine wildlife prior to planning further research. Our review aims to summarize information gathered from petroleum toxicity and determine what can be applied to diluted bitumen toxicity. The focus will be on marine vertebrates, specifically mammals, reptiles, and to summarize and update a 2015 review on fish. Effects consist of both lethal and sublethal endpoints including gene expression, physiology, and fitness. A search was conducted on the Web of Science database, using search parameters for papers from 1900 to 2018, with no filters applied. Papers examining a single component of petroleum (e.g. pyrene, cadmium) were excluded from the search. The majority of papers were on effects of oil spills, as well as laboratory studies conducting exposures of PAHs, components of petroleum, or a petroleum product on marine vertebrates. The results of this literature review will help inform academics, researchers, and policy makers on how to better protect B.C.'s marine wildlife from oil spills.

**WP153 Developing reference materials for advancing chemical and toxicological characterization of soluble organics in oil sands process-affected waters**

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Advanced separation and high resolution mass spectrometry methodologies have allowed for significant advancement in the understanding of the chemical composition of the complex organic mixtures within oil sands process-affected water (OSPW) and other bitumen-influenced waters over the past 10 years. However, quantitative assessment of the mixture components has remained unreliable due to the absence of relevant reference materials. Quantitative analysis of these complex organic mixtures is vital for monitoring and reclamation research initiatives as currently there are no definitive means to accurately establish water quality guidelines from toxicological assays nor can the quantity of tailings migration be accurately assessed. To address this need, current research is underway to develop certified reference materials (CRM) for use in chemical and toxicological characterizations of OSPW-derived organics. These CRMs include synthesized individual reference compounds as well as comprehensive mixtures of extracted OSPW-derived soluble organic compounds. Recent collection of active OSPW samples was coordinated through Canada's Oil Sands Innovation Alliance (COSIA) and provided >100L water from each of 9 different active OSPW containments, with several collections taking place over multiple locations throughout the sampled tailings pond. This variable sampling will allow for the creation of a comprehensive CRM mixture of soluble organics found within active OSPW containments, and will also allow for a thorough investigation of the chemical variability that exists within and between OSPW sources.

**WP154 Naphthenic Acid Diagnostic Ratio Analysis; a New Approach for Identification of Alberta Oil Sands Process-Affected Water and Environmental Monitoring**

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A new approach to environmental monitoring of the naphthenic acids, sourced from oil sands tailings containment ponds, is proposed using relative percent difference ratios. The oil sands process water (OSPW) tailings are high in these persistent organic compounds that have been under review because of their high toxicity. The proximity of the ponds to waterways and aquafers is a source of environmental concern, and there is a need for procedures that distinguish anthropogenic seepage from natural sources of naphthenic acids. The authors propose a novel biomarker ratio analysis of data collected during LC/QToF quantitative analysis. The analytical method identifies compounds to ≤5 ppm mass accuracy by formula and was adapted to include heteroatomic sulfur containing homologues. Responses for selected biomarkers were compared in ratio by relative percent difference, in a similar manner to that relied upon internationally for forensic oil spill analysis. These diagnostic forensics allowed for the comparison between duplicate samples both graphically and by relative %Difference. No interferences have been observed for surface and groundwater control samples. Based on the currently selected biomarker ratios, OSPW samples from different industrial sites could be identified as a definitive "no match" result, while those from within one industrial site were a "match". Temporal OSPW samples taken at different times from the same site showed a "match" over the short term but not over one year. The rapidity of this diagnostic ratio calculation and simplicity of visual presentation, in addition to its ability to set values on the relative %Difference results for "match", "probable match", or "no match", make it an excellent potential tool for regulatory purposes. Observed relative %Differences for currently selected biomarkers will be discussed, with the aim of proposing a new procedure for the future environmental monitoring of naphthenic acid seepage, spill, or breach.

**WP155 Evaluating the roles dissolved and particulate fractions of eroding natural oil sands in Athabasca Rivers using water accumulated fractions**

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In the tributaries and mainstem of the Athabasca River in the oil sands region, inputs of bitumen-derived constituents from natural oil sands as well as potential contaminants associated with mining activities will affect water quality, thus raising concerns of cumulative impacts on aquatic organisms. The partitioning of bitumen-derived constituents into air, water and sediment during natural erosion influences the exposure to and effects of constituents on aquatic organisms. There is a need to better understand the environmental impacts associated with bitumen-derived constituents as a function of erosional processes in order to address the issue of potential cumulative impacts. The objective of this study was to isolate the dissolved and particulate fractions of bitumen ore mixed with water and evaluate the toxicity of the individual and combined fractions on the survival of *Hyalella azteca*. Two bitumen ore samples were evaluated; 1) bitumen ore collected from a McMurray Formation exposure in the MacKay River valley and 2) bitumen ore collected from an open pit mine and stored at the InnoTech Sample Bank. Bitumen ore was mechanical mixed for 24 h in water to simulate erosion, and then filtered to separate the dissolved and particulate fractions. Toxicity tests were conducted using a 96-h *H. azteca* survival test at bitumen concentrations of 0-2.5 dry wt g/L for four different treatments: bitumen ore (unmixed), bitumen ore (mixed), dissolved fraction and particulate fraction. The results for both bitumen ore samples indicate significantly higher toxicity for all treatments that contain particulates relative to the dissolved fraction. Water chemistry analyses of metals and polycyclic aromatic compounds (PAC) were used to estimate threshold concentrations. Threshold concentrations were compared with available data on environmental concentrations of metals and PACs in suspended sediments collected from the Athabasca River. Interestingly, within the age range (2- to 9-day old) of neonates recommended for testing (Environment Canada, 2013), it appears that younger neonates (2- to 4-day) are more sensitive to fine particulates than older neonates (7- to 9-day old) in 96 h water exposures. The findings of this study are important to establishing a comprehensive environmental monitoring program and developing an effective watershed management strategy in the oil sand region.

**WP156 Comprehensive two-dimensional gas chromatography mass spectrometry for the analysis of substituted and unsubstituted polycyclic aromatic compounds**

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Alkylated polycyclic aromatic compounds (APACs) are a complex class of environmental contaminants that are ubiquitous and known to be toxic. Each PAC group consists of multiple compounds and the number of theoretically possible isomers can reach into the thousands. Currently, each PAC group is quantified collectively as a single group of compounds. However, individual PACs can reveal important information on how the PACs were formed and this information may be used to determine sources of PACs in samples, potentially even for similar PAC sources. To date, one-dimensional gas chromatography (GC) has been used to characterize the profile of these compounds in the environment. It is hypothesized that many of the limitations with the 1D GC approach to characterizing APACs can be circumvented using GC×GC. Here we apply comprehensive two-dimensional GC coupled to high-resolution time of flight mass spectrometry (GC×GC-HFTOF/MS) to aid in the separation, identification and quantitation of APACs in three environmental matrices: mussel tissue (*Mytilus edulis*), lubricating oil and coal. In the absence of authentic analytical standards, differences in the mass spectral fragmentation pattern of isomers were used to confirm the identity of isomers within a PAC group. A detailed diagnostic characterization of each PAC grouping in the 3 environmental samples is presented. The GC×GC-HFTOF/MS method was validated according to the EURACHEM guidelines and used to quantify a biological standard reference material (SRM 2974a) from the National Institute of Standards and Technology. The method met all the standard method performance requirements such as trueness, precision and measurement of uncertainty and is fit for quantifying these compounds in biota. Furthermore, the method was used to identify and quantify additional PAC compounds in the SRM 2974a material which to date have not been certified. With appropriate statistical analytical tools, the described GC×GC method can be used as a tool for more robust source fingerprinting and source apportionment of PACs in the environment.

**WP157 Identification of Halogenated Polycyclic Aromatic Compounds in Biological Samples from Alberta Oil-Sands Region**

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Halogenated polycyclic aromatic compounds (HPACs) were identified in biological samples from the Alberta Oil-Sands Region (AOSR) using gas chromatography high-resolution time-of-flight mass spectrometry (GC-HRTOF-MS) at a resolving power of 25,000. Knowledge of the electron ionization (EI) fragmentation behavior of individual HPAC isomers, achieved by injecting authentic standards in full-scan MS mode, was paramount in identifying a suite of HPACs in samples from the AOSR. Numerous compounds were detected in the high resolution total ion chromatogram in the extracts of 4 biological species from the AOSR: river otter livers (*Lutra Canadensis*) and muscle tissue of northern pike muscle tissue (*Esox lucius*), snails (*Gastropoda*) and lake whitefish (*Coregonus clupeaformis*), many of which remain unidentified. However, careful examination of the high-resolution accurate mass data and retention times ($r_t$), suggest that 3 HPACs were positively identified in our AOSR samples. Dichloro-anthracene/phenanthrene, bromo-anthracene/phenanthrene and dibromo-fluorene were identified based on their $r_t$ and the measured mass accuracy (± 3ppm) of 2 characteristic ions prominent in their EI mass spectra. Lipid corrected concentrations of the mono- and dibromo-PACs were estimated to be *ca*. 170.5 and 111.4 ng/g in snails while concentrations of the dichloro-PACs was significantly smaller in lake whitefish and river otter.

**WP158 Determination of Polycyclic Aromatic Hydrocarbons in Surface Water using Simplified Liquid-Liquid Micro-Extraction and Pseudo-MRM GC/MS/MS**

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants found throughout the environment, formed from natural and anthropogenic incomplete combustion such as forest fires and burning of fossil fuels. The monitoring of PAH concentration in surface water is a crucial part of water quality surveillance programs implemented by government agencies. In cases of accidental oil spills, PAH analysis is an important tool for rapid spill source identification and environmental impact assessment. The solubility of PAHs in water is very low; therefore extraction typically requires a complex procedure with large volumes of water and extraction solvent to achieve trace level detection and quantitation. This liquid–liquid micro-extraction (LLME) GC/MS/MS approach sought to simplify the classic liquid-liquid extraction technique and optimize instrument performance for analysis of 18 PAHs in surface water. This LLME method utilizes a pseudo multiple reaction monitoring (PMRM) mode, a technique in which the third quadrupole monitors the same m/z as for the first quadrupole precursor ion. The use of helium as the only collision gas improved the sensitivity by significantly reducing both PAH compound fragmentation and baseline noise. For PAH determination, the PMRM approach proved superior to the classical quadrupole MRM technique in terms of enhanced sensitivity. With observed improvements in sensitivity, micro-extraction using only 4 mL of a novel binary solvent became possible, with corresponding reduction in time consuming sample preparation procedures and toxic solvent usage. Quantifying and qualifying ions, in addition to retention times, were used to verify trace level PAHs. During method validation, the limit of quantitation (LOQ) in surface water was observed to be 10 $ngL^{-1}$ for the target PAHs. The recovery of individual PAHs was in the range of 80 to 114% from a surface water matrix, with a corresponding precision between 1.4 and 4.8% RSD. The robustness and accuracy of this method was demonstrated by its success in repeated proficiency test studies and during routine use in environmental sample testing. Analysis of chemically enhanced water accommodated fraction (CEWAF) samples from toxicological LC50 bioassays has proven its applicability in the presence of crude oil and oil spill dispersants.

**WP159 Source identification of nitrogen heterocyclic polycyclic aromatic compounds in snow, sediment, and air samples from the Oil Sands Area of Alberta**

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The Athabasca Oil Sands (AOS) deposits located in Northern Alberta, Canada, are considered the world's third largest oil reserve. Long-term data sets on major contaminants, such as polycyclic aromatic compounds (PACs), have been gathered in the past few years as part of the Alberta-Canada Joint Oil Sands Monitoring program, initiated in 2012. Potential sources of PACs in the AOS area include natural erosion of geological formations, forest fires, bitumen upgrading, diesel combustion, and airborne dust from roads and mining operations. Focus has been limited on a list of mostly unsubstituted PACs, even though over 200 sulphur-containing PACs were recently identified in AOS environmental samples. This study was designed to explore nitrogen heterocyclic PAC isomers (NPACs) currently not being monitored in snowpack, lake sediment and passive air samples collected at varying distances from the main AOS developments during 2014-2015. Additionally, source samples including petroleum coke (pet coke), haul road dust, and unprocessed oil sands were also analyzed. All samples were analyzed using two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC/ToF-MS). Over 200 NPACs were identified and classified into at least 18 isomer groups, including *c1*-carbazoles, *c2*-carbazoles, *c3*-carbazoles and benzocarbazoles, based on their mass spectra and their location in the 2D-chromatogram. Relative concentrations of the NPACs decreased with distance from the main developments, and with increasing depth of lake sediments, but were detected at least 50 km of the major developments. Additionally, pet coke had a more distinct distribution compared to the road dust and unprocessed oil sands ores and was similar to the near-field environmental samples. These results suggest that pet coke is a potential source for the identified NPACs in near-field sites, and that these compounds have the potential to be used as source indicators for future research in the AOS area.

**WP160 Source apportionment of PAHs in snow and lake sediments in the Athabasca oil sands region using stable and radiocarbon isotopes**

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Previous work has demonstrated the potential of compound-specific stable and radiocarbon isotope analysis to delineate sources of polycyclic aromatic hydrocarbons (PAHs) in Canada's oil sands region [1-4]. In this study, compound-specific stable carbon ($\delta^{13}C$) and hydrogen ($\delta^{2}H$) isotope analyses were carried out on PAHs in sediment cores collected from two lakes in the Athabasca oil sands region (AOSR): one located close to the geographic centre of surface mining operations, and the other situated close to a large steam-assisted gravity drainage (SAGD) facility. Compound-specific isotope ratios of PAHs and radiocarbon ($^{14}C$) contents of solvent-extractable organics in snow particulates deposited on the ice-covered surfaces of fourteen lakes across the AOSR were also determined to quantify the contribution of anthropogenic dust transported directly to these ecosystems. Radiocarbon isotope ratios indicated a significant contribution (up to ~ 80%) of petrogenic or fossil (i.e., $\Delta^{14}C$ = -1000‰) carbon in snowpack dust at some sites. More negative $\Delta^{14}C$ values were found in samples closer to the geographic centre of AOSR mining operations and containing higher levels of particulate matter and PAHs. Stable carbon and hydrogen isotope ratios pointed to petroleum coke (petcoke) as a major source of PAHs (over 90% contribution at several sites) at lakes closest to the mining operations, with forest fire contributions becoming increasingly more significant (up to 100%) at greater distances. The information obtained by isotopic analysis provides the high level of quantitative PAH source apportionment required for effective environmental management practices in the AOSR.

## Evolutionary Ecotoxicology Then and Now: Decades of Progress in the Science of Evolution to Pollutants

**WP161 Can epigenetic transgenerational inheritance be incorporated into ecological risk assessment frameworks?**

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Incorporating transgenerational effects into risk assessment procedures has been previously suggested. However, a review of existing literature indicated that contaminant-induced transgenerational inheritance may be less common than reported. Our literature search identified numerous studies claiming transgenerational impacts, but with little compelling evidence. Therefore, we identified a need for more robust multi-generation epigenetic studies that can adequately evaluate transgenerational inheritance. Specifically, experiments that extend beyond what could be deemed "direct exposure" to F1 and F2 gametes, and also experiments that include subsequent non-exposed generations to evaluate transgenerational recovery times. Also, appropriate experimental replication is required to account for the highly variable nature of epigenetic responses and to address the apparent irreproducibility of current studies. Studies are also needed to correlate epigenetic end points with observable detrimental organism changes before a need for risk management can be properly determined. Furthermore, epigenetics focused studies should include concentrations lower than current "$EC_{10–20}$" or "Lowest Observable Effect Concentrations" for the organism's most sensitive phenotypic end point, as evaluating the effect of higher concentrations is usually already adequately done using more inexpensive endpoints. We propose a regulatory framework and optimal experimental design that enables transgenerational epigenetic effects to be assessed and incorporated into conventional ecotoxicological testing in a more useful manner.

**WP162 Evolved resistance and aryl hydrocarbon receptor pathway gene expression in Gulf killifish (*Fundulus grandis*) from the Houston Ship Channel**

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Wild populations inhabiting chronically contaminated environments may evolve resistance to the adverse effects of toxicant exposure. For example, Gulf killifish (*Fundulus grandis*) populations from highly contaminated portions of the Houston Ship Channel (HSC) have evolved resistance to the teratogenic effects of exposure to some aryl hydrocarbon receptor (AHR) agonists (*e.g.*, PAHs, PCBs). Similar resistance has been found in populations of the closely related Atlantic killifish (*F. heteroclitus*) from highly polluted sites on the Atlantic coast. In both cases, resistance is known to be associated with a recalcitrant AHR. In *F. grandis*, this recalcitrance has only been studied via CYP1A activity. However, it has been shown previously in *F. heteroclitus* that the expression levels of other CYP1 genes are not all similarly reduced in resistant fish. Differences in the relative transcriptional responsiveness of these other AHR pathway genes may play a role in the variability observed among adapted *Fundulus* populations. This study examined AHR related gene expression in *F. grandis* during developmental exposure to AHR agonists. Comparison of the results for reference and resistant populations further characterizes the molecular responses to toxicant exposure that lead to the resistance observed in HSC populations.

**WP163 Metabolomics-DNA methylation cross-talk in killifish from the New Bedford Harbor Superfund site**

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The Atlantic killifish (*Fundulus heteroclitus*) is an estuarine species that are found along the eastern coast of North America. Killifish is considered a good model species to study the adaptive response to chronic exposure to pollutants such as TCDDs, PCBs, and PAHs. Studies have shown that different populations of killifish have adapted to extremely polluted environments, including Superfund sites. One such population is the PCB-adapted population inhabiting the Superfund site at New Bedford Harbor, MA (NBH). Previous studies have shown that this population is resistant to PCB-mediated developmental toxicity. In this study, to identify whether adaptation to PCB exposure has also occurred at the epigenetic level, we investigated the global epigenetics (DNA methylation) and metabolomics and stress response gene expression in brain and liver tissues from the NBH killifish, as well as from a nearby reference site (Scorton Creek, SC). We found significant alteration of several metabolites (such as, choline, betaine, histidine, methioninemyo-Inositol, sn-Glycero-3-phosphocholine etc.) in the NBH killifish compared to the fish from reference site. Furthermore, a marked global DNA hypomethylation was evident in the NBH killifish which is clearly tissue (liver > brain) and gender (female > male) specific. Taken together, we postulate a possible metabolomics-epigenetics (DNA methylation) cross-talk in killifish due to PCB exposure. Possible involvement of this mechanism on the adaptation of killifish to PCB exposure needs to be further investigated. Acknowledgement : This work was supported by the Mid-career Researcher Program (2017R1A2B3002243) through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning.

**WP164 Overview and mechanisms of resistance in the freshwater crustacean *Hyalella azteca***

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*Hyalella azteca* are freshwater, epibenthic amphipods widely used in environmental toxicology to study the impacts of human activities and are highly sensitive to pesticides and metals. However, resistance to a variety of pollutants have been documented in *H. azteca*, where certain populations have tolerance levels 5-1000 fold higher than sensitive individuals. For example, populations with resistance to pyrethroid and organophosphate insecticides have been found throughout urban and agricultural areas of California as well as urban areas of New England. In the chain lakes of the Coeur d'Alene River Basin in northeast Idaho, *H. azteca* have been discovered living in areas contaminated with toxic levels of Pb, Zn, Cd, As, and Cu. Amphipods from this area are also over 100 times more tolerant to Zn than sensitive laboratory populations. The goal of this research is to uncover the molecular mechanisms responsible for resistance in these populations across United States. We are drawing on examples of parallel evolution in insects and crustaceans to show that similar mechanisms of resistance evolve repeatedly. Through these studies, we are also developing molecular assays to identify resistance in natural populations, which will facilitate surveys to understand the prevalence of resistance and its correlation to other indicators of ecosystem impairment.

## Advancing the Adverse Outcome Pathway Concept for Mitochondrial Dysfunction

**WP165 The organochlorine pesticide toxaphene affects fish development and acts as a mitochondrial toxicant**

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Toxaphene is a restricted use pesticide that is produced by reacting chlorine gas with camphene. It was heavily used as a pesticide in the Lake Apopka region in Florida for agricultural purposes in the 1960's and 1970's, and today it remains elevated in the soil despite being banned fifty years ago. The objective of this study is to determine the effects of toxaphene exposure on early life stages of zebrafish, a model fish species used widely for developmental toxicity research. Zebrafish were exposed to 0.011-1.11 µg/L from 6 hours post fertilization (hpf) to 96 hpf. Significant mortality was observed in embryos exposed to toxaphene >1.11 µg/L. Deformities were noted at lower doses (>0.11 µg/L), and included edema as well as spinal and cranial deformities. As ATP production is important for development and may be related to the observed deformities, mitochondrial bioenergetics of 24 hour embryos were assessed following exposure. ZF embryos were exposed to 0, 11.1, or 111 µg/mL toxaphene (0.5% v/v DMSO) for 24 hours starting at the blastula stage (~3 hpf). Oligomycin, the uncoupler Carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone (FCCP), and sodium azide were used to challenge the mitochondria in a stress test. Embryos at 24 hpf were treated with 11.1 and 111.1 µg/L and showed lower basal respiration and lower ATP-dependent respiration compared to vehicle and water controls, suggesting that the capacity of the mitochondria to produce ATP was compromised following exposure to toxaphene. Expression of transcripts related to oxidative stress and apoptosis were also tested and normalized gene expression for caspase 3, caspase 9, superoxide dismutase 1 and superoxide dismutase 2 were not changed in relative abundance. However, heat shock protein 70 was significantly induced (more than 50-fold) in the highest treatment group, suggesting that the embryos were under a significant general stress response. We propose that altered bioenergetics in early development can result in delayed hatch and compromised survival of wild species, and studies are underway to invertigate indigenous populations of fish.

**WP166 Toxicity assessment of Tebuconazole in zebrafish (*Danio rerio*) embryos and larvae: Focus on mitochondrial bioenergetics and activity**

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Triazole fungicides are increasingly used in areas of the US to combat mold and fungi, in order to protect vegetables, citrus, ornamental plants and field crops. Tebuconazole is one such fungicide which is considered to be relatively non-genotoxic, however the EPA classifies this compound as a putative human carcinogen. To learn more about the potential adverse effects associated with tebuconazole in non-target aquatic organisms that inhabit areas in which agricultural run-off occurs, we exposed early life stage zebrafish to the fungicide. Zebrafish embryos at 6 hpf were exposed to 2.5, 25, 50, 100 µM tebuconazole in 0.1% v/v DMSO for 24 hours and mitochondrial performance was evaluated. Twenty-four hour old embryos treated with 100 µM showed lower basal respiration and lower spare capacity compared to vehicle controls, suggesting that the capacity of the mitochondria to produce ATP was compromised following exposure to tebuconazole. In addition, survival decreased in a dose dependent manner over 96 hours. For behavioral assays assessing dark photokinesis (0.1, 1 and 10 µM tebuconazole), 10 µM tebuconazole showed a soporific effect on larval locomotor behavior while in the light/dark preference test (0.01, 0.1 and 1 µM tebuconazole), there was some evidence that the fish spent more time in the dark, suggesting less anxiety. Future studies are focused on identifying the specific mechanisms that may explain altered bioenergetics and behavioral phenotypes following exposure to tebuconazole.

**WP167 Mitochondrial toxicants: Will parental exposure during development lead to later life and heritable effects?**

*C. Bergemann, D.F. Mello, Duke University / Nicholas School of the Environment; C. Shoben, Duke University / Biology; L. Perez, Duke University; L.R. Baugh, Duke University / Biology; J.N. Meyer, Duke University / Nicholas School of the Environment*

Chemical exposure during development can have dramatic and sustained impacts, especially if the chemicals target the mitochondrial electron transport chain. Altered mitochondria function can contribute to the initiation of metabolic and degenerative diseases. However, few studies have isolated the effects of chemical exposure during development (and prior to conception) on organisms later in life and in offspring. In this study, we used the model organism *C. elegans* which has many advantages: a fully sequenced and annotated genome, a short and well-characterized reproductive cycle, and the ability to test many individuals at once. We hypothesized that pre-conception exposure to rotenone, a widely-used pesticide and piscicide that inhibits mitochondrial respiration complex I, would result in lifelong effects and would impact the offspring. For experiments, the parental generation (P0) were exposed to rotenone (0.25 and 1 µM) or vehicle (1% DMSO; control) for 48 hours from egg hatch to the L4 larval stage. Post-exposure, animals were transferred to plates for another 48 hours and allowed to lay eggs. Preliminary data showed that after 48-hours of exposure, the P0 generation had a dose-dependent decrease in growth that continued into adulthood at 96 hours. Functional respiratory measurements in whole nematodes with the Seahorse XFe24 revealed a dose-dependent decrease in the basal oxygen consumption rate (OCR; -29.4% and -50.26%) and non-mitochondrial OCR (-19.4% and -67.7%). There was also a trend for a decrease in mitochondrial spare capacity, basal mitochondrial, and ATP linked OCR. In the progeny (F1), growth and respiration at 48 hours showed no difference between offspring from exposed and control P0 animals. Mitochondrial and nuclear DNA copy number ratio remained unaltered in either the P0 or F1 generation in all treatments. These preliminary data suggest that mitochondrial function is impaired in parental (exposed) animals but recovers in the next generation based on the parameters analyzed. Future experiments will include other mitochondrial toxicants, transcriptomics and epigenetic analysis, as well as the effect of modified caloric intake.

**WP168 Adverse effects of chemical contaminants on neurodegeneration in vitro**

*O. Odia, Texas Tech University / TIEHH / Department of Environmental Toxicology; A. Martinez, E. Smith, Texas Tech University / TIEHH*

Numerous causes of neurogenerative diseases have been associated with environmental contaminant exposures, however, due to its complex etiology, it is difficult to distinguish general neurotoxicity effects from specific neurodegenerative effects. Also, from an invitro perspective, the use of neuron astrocyte, co-cell culture to study neurodegeneration is deserving of further study since this is more realistic than contaminant exposures to a primary neuron culture in addition to the protective role of the astrocyte in normal invivo conditions. In this study, a neuron-astrocyte co-culture was used in as a realistic method to evaluate the effects of chemical contaminants on neurodegeneration from exposures of the neural stem cells to fully differentiated astrocyte neuron coculture in a model that simulates a 3 D exposure format.

**WP169 Investigating mechanisms and consequences of chemical-induced mitochondrial DNA mutagenesis**

*T. Leuthner, Duke University / Integrated Toxicology and Environmental Health; J.N. Meyer, Duke University / Nicholas School of the Environment*

Over millions of years, mitochondria have evolved careful coupling of internal chemistry with external environment to provide energy and important signaling molecules required for development and survival across many forms of life. This depends in part on the function of proteins coded in the mitochondrial genome (mtDNA). MtDNA has long been studied across fields including population genetics, evolutionary biology, and more recently, medicine and toxicology. However, the processes that regulate which mitochondrial genomes are transmitted across generations are not fully understood. I aim to identify potential homeostatic processes in the cell that help organisms defend against mtDNA damage from genotoxicants that cause mtDNA mutations that could have significantly deleterious effects on organism and population health. To do so, I exposed the model nematode, *Caenorhabditis elegans*, to various concentrations of ubiquitous pollutants and known carcinogens: cadmium (Cd) and aflatoxin $B_1$ ($AFB_1$). There is a significant dose-dependent increase in the number of lesions in the mtDNA in both Cd and $AFB_1$ treated C. elegans: from 0.05 to 0.3 lesions/10kb (1mM and 50mM of Cd respectively) and from 0.25 to 0.5 lesions/10kb (5mM and 25mM $AFB_1$ respectively). I also observe a dose-dependent decrease in fecundity. Additionally, there is a significant exacerbation of mtDNA damage in *C. elegans* mutants that are deficient in processes involved in the selective removal of damaged and defective mitochondria: *pink-1* and *dct-1* (two-fold and 1.5-fold, respectively). There is also a significant reduction in the fecundity of the mutants compared to wildtype *C. elegans*, suggesting increased susceptibility to toxicity potentially mediated via mitochondrial dysfunction. Together, the mtDNA damage and reproduction results suggest that these processes may be involved in the removal of mitochondria that have accumulated mtDNA damage. We are currently performing targeted sequencing of mtDNA in *C. elegans* after exposure to Cd and $AFB_1$ to investigate whether or not these mitochondrial homeostatic processes are necessary for removal of mtDNA damage prior to accumulation of mtDNA mutations. This is important to understand because the “footprints” that chemicals leave via specific changes in mtDNA sequences could be developed as biomarkers as well as in risk assessment, since mtDNA markers are widely used in predicting health and measuring genetic diversity of populations.

**WP170 Adverse Outcome Network Development for Acetylcholinesterase Inhibition in Zebrafish (*Danio rerio*)**

*K. Conrow, Arizona State University; N. Vinas, US Army / Engineer Research and Development Center; K.H. Watanabe, Arizona State University / School of Mathematical and Natural Sciences*

Acetylcholine (ACh) is a diverse neurotransmitter that is involved in many processes from cognition to muscle activation. The enzyme acetylcholinesterase (AChE) hydrolyzes ACh in order to eliminate it from the body, and when AChE is inhibited ACh levels increase. An excess of ACh at cholinergic synapses overstimulates both muscarinic- and nicotinic-receptors. These receptors are found in most organs in the body, thus the effects of AChE inhibition can result in multiple adverse outcomes affecting a wide variety of functions. Moreover, a wide variety of chemicals including organophosphates, carbamates and some high nitrogen compounds can inhibit AChE. AChE inhibition has a large impact, but relatively little research has been focused on developing related adverse outcome pathways (AOPs) or a network for this molecular initiating event. This presentation focuses upon the construction of adverse outcome pathways for acute symptoms of AChE inhibition in zebrafish (*Danio rerio*). Using a comprehensive literature review, we identified key upstream events essential to the development of specific pathophysiologies that include seizures, altered retinal structure, and paralysis. Applying those studies, we constructed key event relationships for the development of an AChE inhibition AOP network including identification of data needed for development of quantitative models. Preliminary AOP network results will be presented with references supporting each relationship so that we can more fully represent the wide reaching effects of AChE inhibition as a molecular initiating event.

## Ecotoxicology of Amphibians and Reptiles

**WP171 Spadefoot toads (*Spea multiplicata*) as ecological receptors for metals from uranium mine containment ponds**

*B.K. Kunz, D. Cleveland, J. Hinck, USGS / Columbia Environmental Research Center*

Containment ponds associated with breccia pipe uranium mines in the Grand Canyon watershed create surface water habitats in otherwise arid areas. These habitats attract a variety of wildlife species, including breeding spadefoot toads (*Spea multiplicata*), which opportunistically use the ponds for oviposition and larval development. Because containment pond waters contain elevated concentrations of uranium and co-occurring metals (e.g., copper, cobalt, nickel, and arsenic), *S. multiplicata* are potentially exposed to these metals for the duration of the aquatic larval period. However, it is unknown whether these chronic exposures detrimentally affect the larvae. Previous USGS studies have identified copper and cobalt as toxicity drivers for invertebrates exposed to containment pond water, but there is a general lack of ecotoxicological data on metals for *S. multiplicata*. It is also unknown whether metals bioaccumulated by *S. multiplicata* larvae are transferred into terrestrial food webs when the toads metamorphose. We conducted three types of exposures with *S. multiplicata* to evaluate the chronic toxicity and accumulation of containment pond metals by amphibians: 1) a 10-day exposure to field-collected water from a containment pond at the Canyon Mine (Arizona, USA), 2) a 10-day exposure to field-collected sediment from the same pond, and 3) 44-day single-metal exposures to copper or cobalt that lasted the duration of the larval period. All tests were conducted with newly hatched larvae. Endpoints were *S. multiplicata* survival, growth, development, and body burdens of metals. This study helps predict the consequences of breccia pipe uranium mining on native amphibian populations, and determine the potential for toad-mediated transfer of metals from mine containment ponds into surrounding habitats.

**WP172 The combined effects of pesticides and fertilizer on amphibian stress and bioaccumulation in a terrestrial environment**

*R. Van Meter, Washington College / Biology and Environmental Science; R. Adelizzi, Washington College; D. Glinski, Dublin City University*

Tank mixtures, in which multiple agrochemicals are labelled for combined application to a crop, are popular within the agricultural community because they are time- and cost-effective. However, these applications leave non-target organisms, such as amphibians, at risk of exposure to numerous, potentially harmful chemicals at the same time. Here we explore the effects of a common herbicide (atrazine and alachlor) and fertilizer (urea) tank mixture on juvenile frog stress levels, acetylcholinesterase (AChE) biomarker analysis, as well as bioaccumulation. Southern leopard frogs (*Lithobates sphenocephala*) were reared from eggs through post-metamorphosis at Washington College, Chestertown, MD. At 30-90 days post-metamorphosis, 96 frogs were placed individually in terrestrial microcosms sprayed with an individual chemical or tank mixture for the duration of an 8-hour exposure. Following exposure, all frogs were transferred to clean, aquatic microcosms for 1-hour to obtain corticosterone samples followed by euthanasia. Brain tissues were obtained from all 96 frogs to evaluate acetylcholinesterase then whole-body tissue homogenates were analysed for bioconcentration. Atrazine had a significant effect in increasing corticosterone stress levels among frogs, particularly when combined with alachlor and urea ($p< 0.01$). Stress levels were highest among frogs in the combined atrazine, alachlor and urea tank mixture treatment. Atrazine increased AChE levels ($p< 0.01$) among exposed frogs while urea decreased AChE ($p=0.01$), although no interactive effects of chemical combinations emerged. Bioconcentration of atrazine in frog tissues was 64% greater in the tank mixture containing all 3 compounds as compared to the individual atrazine treatment and alachlor bioconcentration was 54% greater in the full tank mixture relative to the alachlor treatment alone. Agrochemicals can lead to altered stress and impaired physiological responses in amphibians, and when in combination their effect is not easily predicted. These biological changes may limit reproduction, immune response, predator avoidance and many other critical physiological functions. Although challenging, an improved understanding of the effects of co-exposure to environmental contaminants in amphibians is important for their conservation.

**WP173 Investigating the Effects of Agricultural Land Use on Wood Frog (*Lithobates sylvaticus*) Habitat in the Prairie Pothole Region – Northern Great Plains**

*G. Ruso, University of Saskatchewan; C.A. Morrissey, University of Saskatchewan / Biology; N.S. Hogan, University of Saskatchewan / Department of Animal and Poultry Science and Toxicology Centre; C. Sheedy, AAFC / Trace Residue Analysis and Immunochemistry; T. Jardine, University of Saskatchewan / School of Environment and Sustainability and Toxicology Centre*

The wood frog (*Lithobates sylvaticus*) is a wide-ranging species common to the prairie pothole region and may serve as an indicator species for aquatic ecosystem health. Large-scale changes in land use, such as conversion to agriculture, present challenges to amphibians in terms of habitat degradation and pollution as wetlands may act as sinks for contaminants. To investigate the effects of agricultural land use on wood frog habitat we measured various indicators of wetland state, assessed wood frog abundance, and tested water samples for a suite of pesticides from five study areas, two reference and three agriculture-dominated, in central Saskatchewan, Canada in 2017 and 2018. Despite regular application of pesticides, detection frequency was low in all study areas, perhaps owing to below average rainfall. Seventeen of the 24 tested wetlands had pesticides present, but of the 166 pesticides scanned for, only 14 were detected. The most commonly detected pesticides were the herbicides MCPA and 2,4-D, and the neonicotinoids imidacloprid and clothianidin, with higher detection rates in the three agriculture-dominated areas. Surveys for wood frogs found that only 23 of 71 wetlands had positive visual or audible detection, with just 16 containing egg masses or tadpoles. This suggests that wood frogs may not be as prevalent in these geographically-isolated wetlands as was initially thought based on anecdotal evidence, and may serve as an example of uneven distribution of the species across its large range or be the result of variable environmental conditions. To further examine patterns and causes of wood frog rarity, we used eDNA to 1) validate field surveys for wood frog presence and 2) detect the pathogen ranavirus in these wetlands as a potential causal agent. Principal component analysis will be used to assess the influence of all measured variables, including water chemistry, pesticides, habitat, and disease presence data, on wood frog occurrence in this region.

**WP174 The Development of Anuran Prey-Orientation Behaviors as Indicators of Exposure**

*K.r. Scarlett, Baylor University / Environmental Science; P.W. Cusaac, K. Dudley, J.B. Belden, Oklahoma State University / Integrative Biology; C. Pope, Oklahoma State University / Physiological Sciences; S.T. McMurry, Oklahoma State University / Integrative Biology*

Prey capture in toads consists of a stereotypical series of behaviors beginning with orienting toward the prey item ('prey orientation'), followed by pursuit and striking. The goals of this study were to characterize prey-orientation behaviors and develop an assay for use as an indicator of toxicity from toxicant exposure. We first examined whether reinforcement of prey-orientation behaviors influenced habituation in both male and female Great Plains toads (*Anaxyrus cognatus*) with three three-minute trials. Preliminary results suggest that orientations decreased significantly from minute to minute (p=0.0059). Given the decrease in orientations over the three separate trials, an experiment was then designed to determine if reinforcing the orientation behaviors would reduce overall habituation. Average number of orientations decreased by 60% after oxon exposure compared to the controls (51 vs 20; p< 0.0001); however, the decrease was not significant until day 15. Therefore, future testing was limited to 14 days and toads were given reinforcements. Further testing was performed where a consistent number of orientations were established using different time intervals along with the presence or absence of a food reward (51 ± 8/minute). Toads were exposed in a preliminary trial to a vehicle (70% DMSO, n=3) or chlorpyrifos oxon (4 mg/kg in DMSO, n=11) and orientations were assessed daily. A larger experiment (n=53) with multiple treatments (Blank control, DMSO control, 4 ug/g 70% DMSO-CPO, and 40 ug/g 70% DMSO-CPO) was later conducted. The average number of orientations/min decreased by 34% (56 vs 37 for 4ug/g) and 63% (62 vs 23 for 40 ug/g) within the first 24 hours after exposure. There was no significant interaction effect between dose and sex on prey orientations (p=0.3373); however, main effects tests suggested that exposure to chlorpyrifos oxon significantly reduced orientations toward the simulated prey item (p=0.0041). Additional post hoc analyses indicated that the higher exposure (40 ug/g) of CPO caused a greater suppression of orientation behaviors compared to the other treatments. Additional tests were performed in an attempt to further measure motor coordination and activity. Further information will be discussed relative to the usefulness of prey-orientation behaviors as indicators of toxicity following exposure.

**WP175 Effects of Atorvastatin on steroidogenesis in *Xenopus laevis***

*J. Johnson, R.J. Griffitt, University of Southern Mississippi / Coastal Sciences*

Micropollutant pharmaceuticals have become an emerging threat to aquatic ecosystems. Atorvastatin is one of the most widely prescribed drugs for adults aged 18-64. Following ingestion, atorvastatin has a low bioavailability of 14%; the remainder of the drug is excreted as the parent compound. Wastewater treatment plants do not effectively remove atorvastatin during the treatment process; studies of conventional wastewater treatment plants show removal rates of ~50%, with resulting effluent concentrations of 1-2 ug $L^{-1}$ reported. Statin drugs inhibit endogenous production of cholesterol by inhibiting 3-hydroxy-3-methyl-glutaryl-CoA reductase from producing mevalonic acid in the mevalonate pathway. The reduction in circulating cholesterol levels may affect steroidogenesis by reducing the levels of estrogen and testosterone produced in the pathway. Due to the increased sensitivity of amphibians to aquatic toxins, we hypothesize that frogs may be significantly impacted by exposure to environmental statins resulting in a reduction of endogenous synthesis of cholesterol, with potential downstream impacts on steroidogenesis. The central hypothesis of this study is to directly address this issue by using chronic exposure bioassays to assess the potential for atorvastatin to cause overt reproductive and developmental dysfunction at critical life stages of wetland amphibians. *Xenopus laevis*were exposed to both chronic low levels (0.2 ug/L) as well as acute high levels (2.0 ug/L) of atorvastatin. Following exposure, blood plasma was analyzed for cholesterol content as well as testosterone and estrogen levels in males and females, respectively. Additionally, qPCR was performed to provide insight into effects on the steroidogenesis pathway by atorvastatin as well as to determine the effects of atorvastatin in circumstances where blood plasma analysis was not possible due to life stage. Acute studies indicate statistically significant reduction in circulating cholesterol during the first 96 hours post fertilization.

**WP176 A quantitative AOP for activation of the Ah Receptor leading to early life stage mortality in amphibians and reptiles**

*J.A. Doering, USEPA / Mid-Continent Ecology Division; A. Schroeder, University of Minnesota, Crookston / Math, Science and Technology; M. Brinkmann, University of Saskatchewan / School of Environment and Sustainability and Toxicology Centre; Z. Currie, University of Saskatchewan / Toxicology Centre; M. Hecker, University of Saskatchewan / School of the Environment & Sustainability and Toxicology Centre*

Differences in sensitivity to chemicals among species and taxa is a major challenge to accurate ecological risk assessments. Most toxicity information is collected from a few model species and little is known about the relationship between the sensitivity of model species compare to non-model species. This is particularly true for amphibians and reptiles. Quantitative adverse outcome pathways (qAOPs) are quantitative, biologically-based models which describe key event relationships that link a molecular initiating event (MIE) to an adverse outcome (AO). qAOPs can serve as a useful tool to determine the relationship between the sensitivity of chemicals with a MIE and an AO among species. Previously, a qAOP had been described for the indirect relationship between activation of the aryl hydrocarbon receptor (AHR) by dioxin-like compounds (DLCs) and early life stage mortality in birds and fishes. Reptiles and amphibians bridge the evolutionary gap between birds and fishes. Therefore, considering the qAOP has shared applicability to evolutionarily divergent birds and fishes, it was hypothesized that it also has applicability to amphibians and reptiles. However, little information was available on AHRs or sensitivity to DLCs in amphibians and no information was available for reptiles. Therefore, this study investigated sensitivities to activation of AHRs (AHR1s and AHR2s) in an in vitro AHR transactivation assay and in vivo early life stage sensitivities for an amphibian, the African clawed frog (*Xenopus laevis)*, and a reptile, the common snapping turtle (*Chelydra serpentina*). Early life stage mortality was assessed in African clawed frog embryos exposed to serial concentrations of one of two DLCs: 2,3,7,8-TCDF or 2,3,4,7,8-PeCDF. Early life stage mortality was assessed in common snapping turtle embryos exposed to serial concentrations of one of four DLCs: 2,3,7,8-TCDF, 2,3,4,7,8-PeCDF, PCB 126, or 2,3,7,8-TCDD. Further, in vitro AHR transactivation assays were used to determine sensitivity to activation of AHR1 alpha and beta isoforms of African clawed frog and AHR1 and AHR2 of common snapping turtle to the selected DLCs. Validating the qAOP linking activation of the AHR to early life stage mortality in birds and fishes for applicability to amphibians and reptiles could guide more objective ecological risk assessments of DLCs to native species of amphibians and reptiles, which are not easily studied.

**WP177 In vitro toxicity of Perfluorooctanic Acid, Benzo[a]pyrene, and Polychlorinated Biphenyl 77 in Loggerhead Sea Turtle Skin Fibroblasts**

*J.D. Pierro, Texas Tech University / TIEHH / Environmental Toxicology; C. Wittmaack, Texas Tech University / Department of Environmental Toxicology; J.G. Surles, Texas Tech University / Department of Mathematics and Statistics; B.M. Higgins, NOAA National Marine Fisheries Service; C.A. Godard-Codding, Texas Tech University / TIEHH / Department of Environmental Toxicology*

Perfluorooctanic Acid (PFOA), Benzo[a]pyrene (B[a]P), and Polychlorinated Biphenyl 77 (PCB 77) are contaminants commonly found in marine environments. These chemicals have been detected in the tissues, eggs, and prey items of sea turtles, including in loggerheads (*Caretta caretta*). These chemicals are known for cytotoxicity, mutagenicity, carcinogenicity, immunotoxicity, adverse reproductive and developmental effects, and negative impacts on pulmonary, renal and hepatic systems in a wide range of species. Their effects in the threatened loggerhead and in other sea turtle species are poorly known since in vivo toxicological testing is prohibited in these animals. Here we report on in vitro cytotoxicity of PFOA, B[a]P, and PCB 77 in primary loggerhead skin cells. We established the primary cultures from skin biopsies collected from healthy animals reared at the NOAA Sea Turtle Facility in Galveston, Texas. The effects of chemical exposure were measured by two common viability assays – MTT and Lactate Dehydrogenase (LDH). Primary cultures (n=7) were exposed to each chemical for 24, 48, 72, and 96 hours. Exposure concentrations were based upon environmentally relevant levels found in sea turtle eggs, plasma, tissue, prey items, and habitats. Toxicant concentrations were 0.01, 0.1, 1, and 10 µM for PCB 77 and B[a]P, and 0.05, 0.5, 5, 50, and 500 µM for PFOA. Data was analyzed using Analysis of Variance (ANOVA) and Tukey's tests, and Kruskal-Wallis and Wilcoxon tests where applicable. Preliminary analyses detected toxicity of PFOA, B[a]P, and PCB 77 at several time points and concentrations. Cytotoxicity was detected by MTT assays at the highest doses following 72 and 96 hours of exposure for PCB 77 and PFOA. Additional MTT and LDH data analyses are underway. To our knowledge, this is the most comprehensive in vitro study to date on organic contaminant toxicity in sea turtle primary cultures obtained from healthy animals. We are currently developing mathematical models to extrapolate these in vitro data across biological organization levels in order to provide novel information applicable to risk assessment techniques on populations of sea turtles.

**WP178 CYP1A induction in Loggerhead (*Caretta caretta*) sea turtle organotypic skin cultures exposed to benzo[a]pyrene and PCB 126**

*C.S. Calvin, Texas Tech University / TIEHH / Environmental Toxicology; B.M. Higgins, NOAA National Marine Fisheries Service; C.A. Godard, Texas Tech University / Environmental Toxicology / The Institute of Environmental and Human Health*

Organotypic culture refers to the culture of thin precision-cut slices of tissue. Several studies have indicated that this *ex vivo* methodology more closely represents in vivo systems than the traditional in vitro cultures that lack a three-dimensional structure and are composed of only one cell type. Organotypic culture is widely used for toxicity testing in pharmacology and medicine but is still novel in wildlife. We previously validated the viability of organotypic skin cultures in the loggerhead sea turtle, both in media with and without fetal bovine serum, for up to 96h. Viability was assessed by three different endpoints: (1) lactate dehydrogenase (LDH) measured in the culture media via a commercially available LDH Assay kit, (2) quantitation of the internal potassium in skin slices by flame atomic absorption spectrometer, and (3) cell culture establishment from skin slice. The six sea turtle species that inhabit the coastal waters of the United States are labeled as either vulnerable, endangered, or critically endangered by the International Union Conservation of Nature Red List. Polycyclic aromatic hydrocarbons (PAHs) and halogenated aromatic hydrocarbons are common marine contaminants and have been detected in sea turtle tissues. Little is known about the potential adverse effects of these chemicals in sea turtles. Organotypic cultures were established from skin biopsies collected from healthy animals reared at the NOAA Sea Turtle Facility in Galveston, Texas. Tissue slices were cultured in media dosed with benzo[a]pyrene (B[a]P) for 4, 24, 48, and 72h, or with polychlorinated biphenyl 126 (PCB 126) for 24 and 72h. The enzymatic activity of CYP1A, a well-established biomarker of exposure for PAHs and planar halogenated aromatic hydrocarbons, was measured using ethoxyresorufin O-deethylase (EROD), methoxyresorufin O-demethylase (MROD), benzyloxyresorufin O-debenzylase (BROD), and pentoxyresorufin O-depentylase (PROD) by spectrophotometry. Preliminary results indicated that CYP1A detection could be detected in the skin organotypic cultures after exposure to B[a]P. Further analyses for both B[a]P and PCB 126 exposure scenarios are underway.

**WP179 Monoclonal antibodies against loggerhead sea turtle IgY isoforms reveal differential contributions to antibody titers & relatedness with other turtles**

*M.L. Rodgers, The University of Southern Mississippi / Division of Coastal Sciences; C.D. Rice, Clemson University / Biological Sciences*

Serum collected from loggerhead sea turtles, *Caretta caretta*, in the southeast Atlantic Ocean was used to purify immunoglobulin Y (IgY) and develop IgY isoform-specific monoclonal antibodies (mAb). Typical IgY has two heavy and two light chains with a total molecular weight of ~180 kDa. Another common isoform of IgY exists as a truncated form of ~120 kDa, known as IgY (ΔFc). Reptiles and birds may produce the 180 kDa IgY isoform, the 120 kDa IgY (ΔFc) isoform (Fab), or both. mAb LH12 was developed against the 66 kDa heavy chain of IgY, mAb LH1 was developed against the truncated heavy chain of approximately 37 kDA, and mAb LH9 was developed against the 23 kDa light chains. mAb LH9 reacted with the light chains of all sea turtles, mAb LH12 reacted with the long heavy chain of all sea turtles within the family Cheloniidae, and mAb LH1 reacted with the truncated form of IgY in both olive and Kemp's ridley turtles. Circulating IgY antibody titers against *M. mycobacterium*, *E. coli*, and *V. parahaemolyticus* were determined by ELISA in 16 loggerhead samples using these mAbs. mAb LH12 detected higher titers than mAb LH1, and mAb LH9 detected the highest titers due to light chains being shared by both IgY isotypes. Going forward, these new additions to the limited tool box of sea turtle-specific reagents are now available to the scientific community examining the health of these animals in their natural habitat, as well as animals in recovery centers and aquariums.

**WP180 Effects of Exposure Area and Dose on Uptake of Hydrophobic Environmental Contaminants by Acris blanchardi via Dry Patch System (DPS) Exposure Method**

*W.H. Mimbs, Oklahoma State University / Zoology; S.T. McMurry, J.B. Belden, Oklahoma State University / Integrative Biology*

Exposure of terrestrial amphibians to environmental contaminants is thought to occur primarily via the dermal route of exposure as opposed to the oral route primarily considered in mammalian and avian species. In order to better characterize this route of exposure and how different skin areas on the amphibian affect uptake, a "dry" dermal patch application method was developed by the authors that removes several potentially confounding factors such as soil properties and amphibian-soil contact time and allows consistent dosing of test substances to different parts of the amphibians. In this study, we sought to examine the differences in peak concentration and time to peak concentration of the contaminants based on the size of the exposure patch used. Five compounds dissolved in acetone were applied (1,200 ng of each compound) to polytetrafluoroethylene (Teflon™) strips measuring either 12 $mm^2$ or 25 $mm^2$ by area and the patch allowed to dry. These strips were then applied to adult *Acris blanchardi* (Blanchard's cricket frog) on either the dorsal or ventral side, and secured using water-proof adhesive bandage material and tissue adhesive. Five individuals were sampled from the dosed population at 0, 0.5, 1, and 3 hours after exposed began, euthanized, their tissue extracted using the QuEChERS method, and the resulting extract analyzed via GC/

MS. Further, a second test using the same test procedures was performed with the 25 $mm^2$ size strips dosed with the five contaminants at either 24 or 48 ng/$mm^2$.

**WP181 Differences in Hydrophobic Contaminant Uptake Between an Aquatic and a Terrestrial Species via Dry Patch System (DPS) Exposure Method**

*W.H. Mimbs, Oklahoma State University / Zoology; S.T. McMurry, J.B. Belden, Oklahoma State University / Integrative Biology*

Exposure of terrestrial amphibians to environmental contaminants is thought to occur primarily via the dermal route of exposure as opposed to the oral route primarily considered in mammalian and avian species. To better characterize this route of exposure and how different skin areas on the amphibian affect uptake, a "dry" dermal patch application method was developed by the authors that removes several potentially confounding factors such as soil properties and amphibian-soil contact time and allows consistent dosing of test substances to different parts of the amphibians. In this study, we sought to examine the differences in uptake of five hydrophobic contaminants exposed to the aquatic *Xenopus laevis* and the terrestrial *Acris blanchardi* based upon the species and whether the patch was applied dorsally or ventrally. Five compounds dissolved in acetone were applied to polytetrafluoroethylene (Teflon™) strips measuring 25 $mm^2$ by area at a rate of approximately 48 ng chemical/$mm^2$ and the patch allowed to dry. These strips were then applied to both individuals of species (Blanchard's cricket frog) on either the dorsal or ventral side and secured using water-proof adhesive bandage material and tissue adhesive. Five individuals were sampled from the dosed population at 0, 0.5, 1, and 3 hours after exposed began, euthanized, their tissue extracted using the QuEChERS method, and the resulting extract analyzed via GC/MS.

## Remediation and Restoration: Assessing and Measuring Effectiveness for Contaminated Sediments

**WP182 Colonization and growth of organohalide respiring biofilms on carbonaceous amendments**

*S. Capozzi, University of Maryland at College Park / Environmental Science; B.V. Kjellerup, University of Maryland at College Park / Civil and Environmental Engineering*

Removal of polychlorinated biphenyls (PCBs) from contaminated aquatic sediments is a priority due to the ability of these persistent organic pollutants to enter the food chain, where they bioaccumulate with toxic and carcinogenic causes as a result. Recent success with reduction of the bioavailability of PCBs due to adsorption onto carbonaceous amendments has led to the recognition of in situ treatment as a viable remediation approach for contaminated sediment. *In situ* microbial degradation of PCBs represents a significant improvement in recent remediation efforts, since previous attempts have largely been unsuccessful due to the low abundance of naturally occurring PCB-degrading microorganisms as well as the use of liquid inoculum for bioaugmentation where free floating microorganisms are easily washed away. To overcome these challenges, this study evaluated the potential for localizing and concentrating PCB-dehalorespiring biofilms onto surfaces of materials varying in material properties (i.e. sorption, conductivity, surface area) and applied these biofilm communities to microcosms amended with Grasse River sediment. Biofilm colonization and growth of *Dehalobium chlorocoercia* DF-1 on coal activated carbon (AC), bone biochar (BC), polyoxymethylene (POM), and sand was monitored over 159-day period. DF1 formed thin and patchy biofilms ranging in thickness from 3.9 to 6.7 µm and the surface area covered with biofilm (i.e. biosurface area) varied for the tested materials. Biosurface area increased steadily over 159 days and showed that highest coverage for coal AC (20.2 ± 4.1%) followed by bone BC (14.7 ± 5.8%), POM (10.2 ± 2.5%) and sand (5.5 ± 1.4%). The biosurface area increased with increasing sorptive capacity thus indicating a preferential attachment on more sorptive materials. In addition, quantitative polymerase chain reaction (q-PCR) results showed a high abundance of DF1 on the various materials after 159 days with bacterial numbers of $6.12x10^9$, $1.53x10^{10}$,$1.17x10^9$, and $3.46x10^9$ gene copies per gram d.w. of coal AC, bone BC, POM and sand, respectively. Thus it was possible to establish organohalide respiring biofilms attached to sorptive materials at high cell densities that dechlorinated PCBs during the experiment. Future work will include assessment of the biofilm inoculum for bioremediation of weathered PCB and if organohalide respiring bacteria embedded within a biofilm can successfully be applied to subsurface environments.

**WP183 Comparative potentials of *Duranta repens* and *Duranta erecta* in nickel and asenic remediation**

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This study assessed the bioremediation potential of two ornamental plants (*Duranta repens and Duranta erecta)* exposed to gradient concentrations of arsenic and nickel. Metal solutions of $As_2O_3$ and $NiCl_2.6H_2O$ at the concentrations of 0, 10, 20, 40, 80 and 160 mg/kg were prepared using analytical grade salts. Data collected at the end of eight weeks after planting (WAP) were analysed using descriptive and inferential statistics. The physical and chemical parameter of experimental soil prior to planting were within the acceptable limit. At 8 WAP, the soil pH values decreased from 6.2 to 5.7 while available phosphorus increased from 34 to 44 mg $Kg^{-1}$ with increase in concentration of arsenic. On the other hand, for nickel, the soil pH increased from 6.6 to 7.1 while organic contents showed a steady increase from 2.9 to 4.8 g $kg^{-1}$ with increase in concentration of nickel. Both *Duranta repens and Duranta erecta* survived across all concentration of both metals in the first two weeks after planting, though *Duranta erecta* experienced faster growth than *Duranta repens.* The growth rate of the two plants began to decline after three weeks after planting at higher concentrations of nickel and eventually led to the death of *Duranta erecta* at 160 mg/kg and *Duranta repens at* 80 mg/kg treatments. For *Duranta repens,* arsenic toxicity was observed in term of retarded growth from 40 mg/kg to 160 mg/kg which led to the death of the plant at 160 mg/kg while *Duranta erecta* survived across all concentrations of arsenic. *Duranta erecta* had a higher root length than *Duranta repens* which was 156.7% and 150% at 20 mg/kg for arsenic as well as 167.4% and 134% for nickel. *Duranta erecta* showed poor bioconcentration factor for arsenic which ranged between 0.85 and 1.2 but showed a good bioconcentration factor for nickel which ranged between 2.2 to 4.7. *Duranta repens* had bioconcetration factor for both arsenic and nickel ranged between 1.1 to 1.4 and 3.6 to 8.0 respectively. *Duranta repens* had transfer factor for both arsenic and nickel while *Duranta erecta* only had transfer factor for nickel. It was concluded that both plants are suitable for phytoremediation of heavy metals. While *Duranta repens* is suitable for phytoextraction of both arsenic and nickel, *Duranta erecta,* is suitable for phytoextraction of nickel but phytostabilizaon of arsenic.

**WP184 Natural amorphous organic matter does not diminish the bioavailability of 2,3,7,8-tetrachlorodibenzo-p-dioxin to mice**

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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is one of the most persistent organic pollutants (POPs) found in soils and sediments. It has been linked to several adverse health outcomes in humans and wildlife, including suppression of the immune system. This contaminant is readily sorbed to soils/sediments due to its extremely low water solubility. Presently, the bioavailability of soil/sediment-associated TCDD to mammals is not completely understood. As demonstrated in our previous studies, TCDD adsorbed to inorganic constituents representing natural geosorbents, i.e. porous silica and smectite clay, exhibited the same bioavailability as TCDD dissolved in corn oil. In contrast, when sequestered by activated carbon TCDD bioavailability to mice was completely eliminated. In this study, we evaluated the effects of natural amorphous organic matter (NOM), in the form of an aquatic humid acid, on the bioavailability of TCDD using the same mammalian (mouse) model. The TCDD (dissolved in DMSO) was mixed with an aqueous suspension of the NOM and with corn oil separately, then administered to mice via oral gavage. Based on its very low water solubility most TCDD is expected to be associated with NOM, most likely by partitioning into a hydrophobic microenvironment within the three dimensional structure of the NOM. The relative bioavailability of TCDD was assessed by quantifying and comparing two sensitive aryl hydrocarbon receptor-mediated responses in mice: 1) hepatic induction of *cyp1A1*mRNA, and 2) suppression of immunoglobulin M (IgM) antibody-forming cell (AFC) response which is an indicator of immunotoxicity. Similar hepatic induction of cyp1A1 mRNA and suppression of IgM AFC was observed for TCDD in the NOM-sorbed form and dissolved in corn oil, revealing no loss of bioavailability due to its association with NOM. Hence, NOM-sorbed TCDD is as capable of suppressing humoral immunity in mice as TCDD dissolved in corn oil, i.e TCDD exhibits 100% relative bioavailability when associated with amorphous NOM. These results indicate that NOM-sorbed TCDD is likely to fully retain its bioavailability to mammals and, by inference, humans.

## High-Throughput Screening and Ecological Risk Assessment

**WP185 Adaptation of methods for the immunofluorescent visualization of thyroxine (T4) in larval fathead minnows (*Pimephales promelas*)**

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Thyroid disruption screening methods in larval fish often include the assessment of growth and thyroid-related gene expression. The direct measurement of thyroid hormones (THs) has also been employed via enzyme linked immunosorbent assays (ELISAs) and liquid chromatography mass spectrometry (LCMS). However, ELISAs often require a large number of animals to be sacrificed and the pooling of tissues to achieve detectable levels of THs whereas the utilization of LCMS calls for expertise in analytical chemistry techniques. In contrast, the thyroxine-immunofluorescence quantitative disruption test (TIQDT), which allows for the fluorescent labeling of thyroxine (T4) within thyroid follicles, requires minimal training, reduces animal use, consumes only low-cost reagents and produces relatively rapid results. As such, the goal of the current study was to adapt the TIQDT for use in the fathead minnow (*Pimephales promelas*), a commonly used fish model for aquatic toxicity testing including thyroid disruption. Fathead minnow larvae were exposed to either a low (25 mg/L) or high dose (70 mg/L) of the model thyroid suppressant, propylthiouracil (PTU), for 30 days beginning at < 24 hours post hatch. At 7 days post hatch (dph), a subset of larvae was sacrificed for the immunofluorescent labeling of T4. Each larva was imaged and integrated density (ID, a metric that accounts for pixel intensity and area) was measured. Relative to control larvae, 4.8 and 4.5-fold decreases in ID were observed amongst low and high dose PTU exposed larvae, respectively. In addition, significant reductions in growth were observed at 7 and 30 dph, and alterations in thyroid-related gene expression (*i.e. deiodinase 2 (di2)* and *transthyretin* (*ttr*)) were observed at 30 dph, further confirming the inhibition of T4 synthesis. The results of this study demonstrate that the TIQDT is a viable method for the assessment of T4 in larval fathead minnows and may be applicable in the screening of potential thyroid-disrupting compounds.

**WP186 High Throughput Screening and Environmental Risk Assessment State of the Science and Emerging Applications**

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In 2007, the US National Research Council (NRC) published a report that clearly identified the lack of toxicity data for most existing chemicals as a major barrier to efficient and cost-effective risk assessment. The authors proposed a path forward that emphasized the use of predictive, high throughput assays to characterize the ability of chemicals to perturb biological pathways critical to health. The scientific community rapidly embraced this vision and major efforts such as the US Environmental Protection Agency ToxCast program were developed to advance the field. While the vision was directed towards human health risk assessment, it was soon understood that it could also be translated to ecological risk assessment. On April 2018 SETAC held a focused topic meeting to evaluate the state of the science with regard to High-Throughput Screening and its emerging applications to environmental risk assessment. Over 100 international participants from academia, government, industry, and other sectors contributed to presentations, demonstrations, and discussions centered on the topic. The meeting provided an opportunity to explore the progress that has been made, as well as the challenges that remain with regard to implementation of the NRC's vision and broadening its scope to encompass a full range of environmental risk assessment contexts. Here, we highlight some of the major topics and discussions from the focused topic meeting with specific regard to major needs for implementation that were also identified by the NRC in 2007, as well as challenges, opportunities, and next steps.

**WP187 In Vitro Screening for Chemical Inhibitors of Human and Amphibian Iodotyrosine Deiodinase Activity**

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Iodotyrosine deiodinase (dehalogenase, IYD) catalyzes iodide recycling from monoiodotyrosine (MIT) and diiodotyrosine (DIT) and promotes retention of iodide in thyroid follicular cells. IYD has an important role in maintaining appropriate levels of thyroid hormones through conservation of this scarce micronutrient and is especially critical for organisms in low iodide environments, including most freshwater ecosystems. Loss of function or chemical inhibition of IYD reduces thyroid hormone synthesis, which leads to insufficiency in tissues and subsequent negative developmental consequences. Our objective was to develop a 96-well plate in vitro assay that could be used to screen chemicals for inhibition of IYD and to compare inhibition between mammals and amphibians. We utilized recombinant human IYD enzyme and *Xenopus laevis* liver microsomal fractions to establish robust assays using MIT as the substrate, 3-nitro-L-tyrosine (MNT) as the positive control, and a non-radioactive detection method. A set of ten chemicals was used to establish the assay, including seven known or suspected inhibitors of IYD (MNT, 3,5-dinitrotyrosine, 3,5-dibromotyrosine, triclosan, phloxine-B, L-mimosine, and bromoxynil) and three suspected non-inhibitors (genistein, dibutyl phthalate, and bisphenol A). Chemicals were tested at a maximum concentration of 200 µM, with seven graded concentrations to produce an inhibition concentration-response curve. Assay performance metrics support use of this assay for screening, with high Z' factor (0.7 or greater) and low variability in the control chemicals (DMSO and MNT). With the recombinant human IYD, five chemicals produced inhibition of IYD ranging from 60-100% (compared to DMSO control), with IC50 ranging from 0.05 µM to >200 µM. There was strong cross-species agreement between human and *Xenopus*, and all chemicals produced comparable maximum inhibition and IC50s, with minor shifts in rank order potency. These assays for inhibition of IYD activity in a 96-well plate format can be used for future screening of large chemical libraries. Additionally, these results indicate that IYD response to potential chemical inhibitors is conserved across vertebrates. *This abstract does not necessarily reflect USEPA policy.*

**WP188 In Vitro Screening of *Xenopus laevis* Deiodinase 3 for Inhibition by ToxCast Chemicals**

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Thyroid hormones are necessary for normal sequential development and metamorphosis of amphibian tissues and organs. Deiodinase enzymes in peripheral tissues are critical components of the system that catalyze the removal of an iodine from thyroid hormones to either activate or inactivate the hormone. We conducted a screening study of chemical inhibitory activity toward amphibian (*Xenopus laevis*) Type 3 iodothyronine deiodinase (DIO 3) enzyme. The chemicals were selected from across the ToxCast Phase 1, Phase 2 and e1k chemical libraries, based on results from previously screening these chemicals against the human DIO 3. Recombinant *X. laevis* DIO 3 enzyme was produced in cell culture and used in a 96-well plate in an initial single-concentration (200 µM) screening assay. Chemicals were further selected for testing in concentration-response mode if found to inhibit the *X. laevis* DIO 3 in the single-concentration screening, as well as having low IC50 values for the human DIO 3 enzyme. Some of these chemicals of interest included antimicrobials Triclosan and chlorhexidine diacetate, and the insecticide Fipronil. Xanthohumol was used as a positive control for DIO3 inhibition. The IC50 values for the human DIO 3 enzyme for these four example chemicals were 61.1, 2.6, 1.2 and 0.3 µM, respectively. The IC50 values for *X. laevis* DIO 3 were 266.3, 9.5, 0.7 and 0.3 µM, respectively. Overall, the data indicate similar activity of the chemicals, likely due to conserved amino acids between these species within the enzymes' catalytic sites. *This abstract does not necessarily reflect USEPA policy.*

**WP189 Incorporating HTS data in classical data based screening risk assessments**

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High Throughput Screening (HTS) data has many applications but such applications to date have mostly focused on molecular toxicology such as Adverse Outcome Pathways (AOPs). Until such time when AOPs are truly curated in detail and ubiquitous is there a way to use HTS data to perform risk assessments? Various methods have been proposed but one of the common drawbacks is the complexity and sheer size of a database such as ToxCast21. In depth familiarity with the various enzyme assays (all 1,000 plus) is required to make a determination of possible mechanistic relations. At greater than 1 billion data points (approximately 10,000 chemicals by 1000 assays by on average > 100 datapoints per assay) when fully populated, ToxCast21 both deserves in depth examination as well as the ability to survey big trends in big data. Verisk 3E decided to provide a simplified recast of ToxCast21 data which is more amenable to interpretation by classical toxicologist and would accelerate the adoption/use of such data within the regulatory community. Rather than displaying test results by assay type first (i.e. lump all stress response type assays together) we first sort by tissue type (e.g. liver). This allows correlation of classical target organ information with HTS data without getting stuck on what a response/hit on XYZ assay really means/implies. Sorting by tissue type truly provides a big picture of big data. High frequency of "hits" for a particular tissue correlates fairly well with target organ information, although additional targets often pop up. Drilling down within tissue types allows one to focus on families of assays e.g. DNA binding. We used Aldrin as an example. Liver hits totaled 36 out of 220 liver tissue assays. Cell cycle, nuclear receptor and DNA binding had the highest hit ratio. Cytokines and cell morphology had relatively low response rates. Other tissue systems with high hit ratios include cervix, skin and vascular. For the latter (not surprisingly) cytokine related assays had the highest hit ratio/response rate. Based on the number of tissues and assay type we developed a risk assessment frame work, which allows numerical scoring of an HTS risk screening estimate. The methodology allows incorporation of HTS data within a classical toxicology based risk assessment screening framework. HTS data can now be used for regulatory screening using the established 3E Green Score methodology.

**WP190 Salmonid pituitary cells as a test system for screening endocrine disrupting compounds**

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The pituitary gland is a central regulator of reproduction, producing two gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which regulate gonadal development, sex steroid synthesis and gamete maturation. We have previously observed that waterborne exposure of salmon and trout to 17α-ethynylestradiol (EE2) and the selective serotonin reuptake inhibitor (SSRI) fluoxetine alter LH beta subunit (*lhb*) and FSH beta subunit (*fshb*) mRNA levels, respectively, in vivo. These results motivated us to expand our studies by developing an in vitro test system using pituitary cells isolated from previtellogenic female coho salmon and rainbow trout. Preliminary studies were performed to optimize culture conditions and to establish the time course of *fshb* and *lhb* gene expression with and without addition of endogenous sex steroids (estrogen [E2] or 11-ketotestosterone). After optimizing assay conditions, a suite of 12 contaminants and other hormones was evaluated for their effects on *fshb* and *lhb* gene expression. Each chemical was tested at 4-5 different concentrations up to solubility limitations in cell culture media. Results indicated more chemicals altered *lhb* than *fshb* mRNA levels. The more potent chemicals were estrogens (E2, EE2) and aromatizable androgens (testosterone), which induced *lhb*. The estrogen receptor antagonist 4-OH-tamoxifen decreased the E2 stimulated expression of *lhb*. Among several SSRIs tested, the sertraline metabolite norsertraline was notable for both increasing *fshb* mRNA levels and decreasing the E2 stimulation of *lhb*. These results indicate that diverse types of chemicals can alter gonadotropin production in fish. Further, we have shown that pituitary cell culture is useful for screening chemicals with potential endocrine disrupting activity and can support quantitative adverse outcome pathway testing. Supported by EPA-STAR grant R835167.

**WP191 ToxCast Results to Identify Chemical Hazards Across Species**

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The US Environmental Protection Agency's (EPA) Toxicity Forecaster (ToxCast™), is a suite of rapid high-throughput screening (HTS) assays used to evaluate chemical safety. Thousands of chemicals have been screened in these HTS assays that cover broad biological space. However, the assays utilized in ToxCast are primarily mammalian-based assays (e.g., human, rat, mouse) as the original intent was to utilize the results to prioritize chemicals based on potential impacts to human health. Recognizing the wealth of information that is available through ToxCast screening results, it is important to consider the utility of these data in understanding chemical effects across species. Therefore, to expand the utility of these data the USEPA's Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS; https://seqapass.epa.gov/seqapass/) tool was employed to evaluate how well the protein targets represented in the ToxCast Assays are conserved across species. For extrapolation of ToxCast results the SeqAPASS tool evaluates protein similarity between protein sequence from the model organism used in the HTS assay and all species with sequence information available, making a prediction of which species are likely similar enough to have the protein target available for a chemical to act upon. This initial understanding of whether the chemical target is available in other species can provide the foundation for extrapolating ToxCast results across species and further identifying where extrapolation is not practical. All 484 ToxCast assays associated with a protein sequence were evaluated with SeqAPASS and made publicly available both through ToxCast and the USEPA's CompTox Chemistry Dashboard (https://comptox.epa.gov/dashboard). Four case studies were developed to describe the SeqAPASS results for ToxCast assay target families including *G-protein-coupled receptors,DNA-binding proteins*, *esterases*, and *growth factors*. These SeqAPASS case studies demonstrate how broadly the HTS assay results can be extrapolated beyond the model organism to understand potential chemical hazard. *The contents of this abstract neither constitute nor reflect official USEPA policy.*

**WP192 What needs to be true: Thoughts on incorporating high-throughput screening data in environmental risk assessments**

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The Environmental Risk Assessment (ERA) framework provides a systematic approach to evaluate the risk of exposure to contaminants in the environment. While the overall framework is generally conserved, ERA practices can differ between countries and regions, as these decisions are based on science but implemented as policy. Some differences include: accepted test species, required testing approaches/guidelines, and the magnitude of applied assessment factors. Differences aside, the quality of the risk assessment is dependent on the availability of data and models for estimating exposure and identifying adverse effects. High throughput (HTP) assays have the potential to not only increase the amount of data available for assessments while reducing the cost and time of generating the data, but also to help develop better predictive models of responses to contaminants. High throughput screening data are globally accessible for thousands of chemicals and models are being developed to help predict potential exposure and effects based on chemical structure and property information. There are, however, a number of challenges to a broad regulatory acceptance for the use of HTP data in ERA. For example, an accessible HTP testing infrastructure is needed in order to support the development and wider use of these new technologies. In addition, HTP data and models have been largely focused on vertebrates/mammals and to date there has not been much focus on identifying pathways and endpoints important in ERA (e.g., algae and invertebrates). Several ways that HTP data could be incorporated into an ERA will be discussed, including: 1) use in compound development, 2) identifying potential modes of action, 3) directly replacing a required toxicity endpoint, 4) refining applied assessment factors, and 5) building and supporting chemical category extrapolation. Several key caveats will be explored and case studies will be used to illustrate challenges and successes in the use of HTP in ERA.

## Developments in Water Quality Monitoring and Analytical Methods in Support of Water Reuse

**WP193 Ion exchange resins and functionalized nanofibres: A comparative study for the treatment of brine**

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Mining, metal extraction, electroplating and desalination processes may result in the release of effluents containing heavy metals and other contaminants into the environment. Desalination processes have resulted in the release of concentrated brine reject, and this poses a threat to the fresh water systems and the environment. Complex brine mixture disposed into the evaporation ponds and other environmental media may experience slow rate of evaporation, due to the presence of the cations such as $Ca^{2+}$ and $Mg^{2+}$. It is therefore imperative to remove these ions to enhance the evaporation rate of the brine mixture. Various methods of heavy metals removal from aqueous solutions such as ion exchange, adsorption, electrochemical treatment, etc. were evaluated. Amongst these, adsorption, a method which depends solely on the functional group(s) on the adsorbent surfaces was investigated. The application of functionalized super hydrophilic materials such as nanofibres as adsorbents to brine treatment, alongside chelating resins in a comparative study of batch adsorption experiment showed interesting characteristics. The surface functionality and morphology/pore structure is characterized using FT-IR and scanning electron microscopy (SEM) respectively, while metal ion concentration determined using the ICP-OES. The adsorption kinetics of the resin and the nanofibre in the adsorption will be studied to assess the performance of nanofibre compared to a conventional commercial resin. The kinetics of the nanofibre-cation interaction is however expected to be sufficiently rapid, reaching equilibrium faster when compared to the chelating resin. The faster kinetics can be attributed to the nanofibre's characteristics such as high porosity, large surface area per unit mass and small interfibrous pore size of the polymer material.

**WP194 Fullerenes in aqueous matrices: An experimental design for analytical method development and validation applying spe followed by LC-MS/MS analysis**

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The fate and effects of emerging contaminants have attracted the attention of the scientific community in the last years, since these compounds have the propensity to cause adverse consequences, even in low concentrations, to the exposed organisms. Endocrine disrupters (EDs), pharmaceuticals, personal care products, flame retardants, surfactants and metabolites, industrial additives, hormones, gasoline additives, pesticides and nanomaterials (NMs) are examples of this class of substances. Nanomaterials (NMs) or nanoparticles (NPs) are present in many consumer products. The massive production and application of NMs justify its presence in several environments. Fullerenes $C_{60}$ and $C_{70}$ are allotropic forms of carbono produced in highly energetic processes of natural origin or anthropogenic sources. In the last years, the increasing application of nanomaterials in several areas of human endeavor besides their physical and chemical properties, contribute for the growth of the global economy. However, the growing production and application of nanomaterials is also promoting discussions about the possible risks of these compounds to the environment and human health. Data have already been reported on the occurrence of fullerenes in different matrices, including the atmosphere, soils and sediments, and fresh water. This study proposes the development, validation of an analytical method for the extraction, pre-concentration and determination of fullerenes in aqueous matrices with the combination chemometrics methods of experimental design for establishing for better analytical parameters of analysis. A complete factorial design was established to optimize the main experimental parameters that affecting the Solid Phase Extraction (SPE) and used in order at achieve a maximum recovery percentage, followed by liquid chromatography – mass Spectrometry (LC/MSMS) efficiency. Preliminary results were the establishment and validation of the analytical conditions for quantification of fullerene in water samples. Our future objective will be to aplly the optimized method to determine these nano-pollutants in water samples from Brazilian aquatic environments.

**WP195 Mobile Phase Additive Selection For Reduction of LC/MS In-Source Fragmentation of HFPO-DA**

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Tetrafluoro-2-(heptafluoropropoxy)propanoic acid (HFPO-DA), commonly known by the trade name GenX for the ammonium salt derivative, is a polyfluoroalkyl ether carboxylic acid introduced as replacement of perfluorinated alkanoic substances (PFAS) fomerly used in various industrial and consumer processes. Environmental monitoring of this compound has increased notably due to recently discovered prior high volume discharge by manufacturers. While liquid chromatography-tandem mass spectrometry (LC-MS/MS) using negative polarity electrospray ionization ($ESI^-$) is the preferred method of analysis, fragmentation of the molecule upon ionization and dimer formation are common observations which inhibit low detection levels in environmental matrices and potential losses in analyte specificity. This work characterized the use of various LC mobile phase additives to control the formation of the bicarbonate adduct $[M+CHO_3]^-$ of HFPO-DA. These included ammonium acetate, ammonium formate and ammonium bicarbonate (2 and 10mM); and formic acid and ammonium hydroxide (0.1 and 0.5%). All additive experiments were performed on a Waters Acquity H-Class equipped with an isolator column in line with pre-injection solvent delivery and a BEH C18 2.1x100 mm 1.7 mm analytical column. Mass spectrometry analysis and source condition optimizations were performed on a Waters Xevo TQ-D operating in both full scan and MRM modes. Adduct identity was confirmed using accurate mass measurement on a Waters Xevo G2-XS QTof. Method reproducibility was assessed by repetition of optimized conditions on additional systems and sites. In monitoring the molecule stability across all mobile phases used, the previously characterized primary fragment generated from the loss of $CO_2$ (-44Da) and additional fragments from breakage at either side of the ether linkage (-161 and -145Da) were assessed relative the parent molecule in full scan where minimal collision energy is applied. Formic acid mobile phase data showed the highest degree of molecule fragmentation. Adduct formation was most stable using the 2 mM ammonium bicarbonate and observed to reduce the degree of fragmentation contribution to overall molecular signal, as well as reduce dimer formation during ionization. An assessment of mobile phase pH from both multiple pH adjustments of ammonium bicarbonate and the inherent pH of all mobile phase compositions showed no strong correlation to in-source fragmentation increase, decrease or relative proportion to parent molecule conservation.

**WP196 Development of an analytical screening method for agricultural chemicals in drinking water using GC-MS**

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In Japan, total demand for water supply has been decreasing year on year because of a declining national population. This resulted in chronic deterioration of financial situation of water facilities and expense to maintain drinking water quality will be reduced. From the view point of chemical analysis, we have to develop cost-effective methods for drinking water quality test. By analyzing chemical compounds using a gas chromatograph-mass spectrometer (GC-MS), we obtain mass spectrum and retention time which are specific for each chemical compound and useful to determine the compound qualitatively. For quantitative analysis, creation of the calculation curve is necessary. If these information, i.e., the mass spectrum, the retention time and the calculation curve, are registered with one database, it will make it possible to analyze various chemical compounds in short time without preparing standard compounds, standard solutions and creating calculation curves. To compose efficient screening analytical methods for drinking water quality test, 176 agricultural chemicals were analyzed with six different GC-MSs using a single analytical method and the precision of the results was verified. When *m/z* which had the strongest intensity was chosen as a monitor ion, the ion agreed with all six instruments in 68% of the targeted compounds. Although the relative standard deviations of each retention time were over 1%, the value was improved to less than 1% by adjusting each relative retention time using corresponding internal standards. The calculation curves were created by one GC-MS which had lowest detection limit. Then, data of other GC-MSs were quantified using this common calculation curves and then the obtained values were compared with setting concentrations. The trueness of over half of tested compounds were within 50 to 200%. However, the values were not good in some compounds. It seems that improvement of the calculation curves is needed to better analyze the agricultural chemicals in drinking water.

**WP197 Monitoring of 176 agricultural chemicals in raw water and tap water by GC/MS screening analytical method**

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In order to ensure the safety of drinking water, many agricultural chemicals in raw water and tap water are monitored by many of water suppliers in Japan. However, Japanese official analytical methods for the agricultural chemicals in drinking water are generally complicated. Much labor and cost is required to apply these analytical methods. Therefore, we need more simple and efficient analytical method for agricultural chemicals. In the present study, we have developed a screening analytical method for agricultural chemicals by gas chromatography/mass spectrometry (GC/MS). Then we applied the analytical method to measure concentrations of agricultural chemicals in raw water and tap water. The target compounds of the present study are 176 agricultural chemicals, which are the "Complimentary Items" in tap water in the Japanese Waterworks Act. We have measured the standards solutions of the agricultural chemicals by GC/MS, then, information of retention time, mass spectrum, and calibration curve was restored to a database. We used anthracene-$d_{10}$, 9-Bromoanthracene, and chrysene-$d_{12}$ as internal standards for GC/MS measurement. The sample preparation method is the same as the Japanese official analytical method released by the Ministry of Health, Labour and Welfare. Then, we have collected 75 raw water and drinking water samples during June to August 2018, and we apply the GC/MS screening analytical method using the developed database. As a result, we have detected 30 agricultural chemicals from raw water samples, 17 agricultural chemicals from drinking water samples. The analytical results by GC/MS screening analytical method were almost the same as those by official analytical methods. Therefore, we judged that the GC/MS screening analytical method we have developed in the present study was applicable to the analysis of agricultural chemicals in drinking water. In order to expand this GC/MS screening analytical method as a standard analytical method of drinking water, we will conduct the validity test of the analytical method among other examination organizations.

**WP198 Monitoring algal toxins in a large reservoir: Relationships with inorganic ions**

*S. Subbiah, A. Karnjanapiboonwong, T.A. Anderson, Texas Tech University / Environmental Toxicology*

Cyanobacteria are widely distributed in fresh, brackish, and ocean water environments, as well as in soil and on moist surfaces. Changes in the population of cyanobacteria can be an important indicator of alterations in water quality. Metabolites produced by blooms of cyanobacteria can be harmful, so cell counts are frequently monitored to assess the potential risk from cyanobacterial toxins. A frequent uncertainty in these types of assessments is the lack of strong relationships between cell count numbers and toxin concentrations. We monitored cyanobacterial toxin concentrations and inorganic ions in monthly water samples from a large reservoir over a 2+ year period, in an effort to determine the existence of any relationships between toxins and common ions. Cyanobacterial toxins were quantified using ELISA (cylindrospermopsin, saxitoxin) or LC-MS/MS (microcystins, anatoxin-A). Common anions and cations were determined by ion chromatography. Anion ($Cl^-$, $NO_3^-$, $SO_4^{-2}$) and cation ($Na^+$, $Mg^{+2}$, $NH_4^+$) concentrations varied by location within the reservoir. In addition, anions ($F^-$, $NO_3^-$) and cations ($Na^+$, $Ca^{+2}$) also varied by season (month). We observed seasonal changes in cyanobacterial toxin concentrations, although toxin concentrations (? microcystins) during the study period never exceeded safety limits. Attempts were made to correlate ion concentrations we measured, and water quality parameters we obtained from an EPA database, to the cyanobacterial toxin concentrations we measured. Results of our study are expected to increase the understanding of potential relationships between human activities in the study location and water quality.

### WP199 Development of a dose-response model for assessing risks from antibiotic resistant *E. coli* in agricultural water reuse

*S. Chandrasekaran, S. Jiang, University of California, Irvine*

Several recent studies have identified antibiotic resistant bacteria (ARB) and residual antibiotics in the effluent of wastewater treatment plants (WWTPs). This is especially true of WWTPs that accept effluent from hospitals or pharmaceutical manufacturers. Moreover, in addition to ARB discharged directly into the environment, the residual antibiotics in the effluent also exert a selection pressure and promote the buildup of additional ARB. This scenario is especially problematic when treated wastewater is used for agricultural irrigation in the effort towards water reuse. There is a potential for the development of infections that do not respond to antibiotic treatments. We here develop a dose-response model taking into account the portion of antibiotic resistant *E. coli* in the treated wastewater in order to understand the magnitude of the threat. In this model, we assume that in the absence of antibiotics, the ARB does not possess increased virulence. This means that the same model used for the antibiotic sensitive bacteria applies for the resistant strain. However, in the presence of antibiotics, through co-ingestion of residual antibiotics and ARB with water or food, the ARB will survive and out compete the sensitive strains. Under such scenarios, the health outcome depends on three factors: the initial bacterial dose of ingestion, fraction of ARB and the concentration of antibiotic. The key contribution of this study is a dose-response model founded in stochastic growth kinetics that accounts for these three factors in determining the health outcome. We demonstrate that the new model outperforms the existing dose-response model for *E. coli* infection in human trial studies. Predictions of human health outcomes are also made in the context of residual antibiotic quantities utilizing kinetic data from in vitro experiments of the ARB to inform the dose-response model.

## Effects of Stormwater Treatment on Receiving Water Quality

### WP200 Advances in permeable pavement technology: Implications for use and reduction of pollutants

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Several green stormwater technologies, including bioretention and permeable pavements have been shown to be effective methods for infiltrating urban runoff, thus reducing flooding. Furthermore, results of a number of studies have shown that certain pollutants contained in stormwater are reduced after passing through specific bioretention systems. Permeable pavements include asphalt and concrete products that allow water to infiltrate into the soil beneath them. As such, they hold promise for the management of stormwater. However, a major flaw with permeable pavements is that they don't have the same durability as conventional pavements. In this study, we evaluated permeable asphalt and concrete that had been modified by adding a carbon fiber product used in the production of Boeing's 787 aircraft wing. Tensile and compressive strength of the modified pavements were compared to conventional permeable pavements. We also evaluated the removal of specific pollutants from stormwater collected from an urban freeway in Seattle. Additionally, a series of aquatic toxicity studies were conducted with untreated urban stormwater and stormwater that passed through conventional permeable asphalt and concrete and carbon-fiber modified permeable asphalt and concrete columns. Results of these studies will be discussed in this talk.

### WP201 Efficacy of compost amended biofiltration swales as green stormwater infrastructure for treatment of toxicants in highway road run-off

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Biofiltration swales, or bioswales, use vegetated soil substrates to filter contaminants from stormwater, decrease sediment load, and reduce erosion. Following a storm, runoff moves slowly through the swale at a shallow depth. While stormwater is retained in the bioswales, pollutants are removed by the combined effects of filtration, infiltration, settling, and biotransformation. The system currently being evaluated uses the addition of compost to further enhance the ability of bioswales to remove toxicants. WSDOT has created guidelines for constructing compost amended biofiltration swales (CABS) and implemented a field test for CABS along Washington State Route (SR) 518 in 2009. As part of an ongoing study, influent and effluent samples are currently being collected at the field site during storm events and tested for metals, PAHs, pesticides, phthalates, and unknowns (LC-QTOF). Acute toxicity and sub-lethal effects of stormwater were also measured using zebrafish (*Danio rerio*) bioassays. Along with researchers from University of Washington (UW) we created a laboratory model for CABS at the Washington State University (WSU) Puyallup Research Extension Center (PREC) to verify field test results in a controlled setting and identify ways that the WSDOT design could be improved. This system is exposed to highway runoff from a previously studied high volume source off SR 520 and tested at different flow rates, swale lengths, and slope gradients. Paired chemistry and toxicology data show how stormwater treatment by CABS differ from traditional soil biofiltration methods. Results presented at Salish Sea show how zebrafish developmental biology is affected by treated and untreated stormwater and how CABS design impacts toxicant treatment efficacy.

### WP202 Sources of fine sediment particles (< 20 µm) in roadway runoff in the Lake Tahoe Basin

*H. Hwang, Texas Southern University / Department of Environmental and Interdisciplinary Sciences; M. Fiala, Texas Southern University / Department of Environmental Science and Technology / Department of Environmental and Interdisciplinary Sciences; R. Wigart, County of El Dorado*

Disturbance within the Lake Tahoe Basin has increased rapidly since the late 1950s primarily due to an extensive road network that boosted urban development and traffic related activities. The clarity of the Lake Tahoe water column has declined since then due to the increased input of fine sediment particles from the Lake Tahoe Basin. This study investigated the contribution of winter road management practices such as abrasive sand application and snow plowing to fine sediment particles (< 20 µm) in roadway runoff that enters directly or indirectly into Lake Tahoe. Roadway runoff was collected from two sites on Pioneer-Trail, a high traffic density road in the southern Lake Tahoe Basin, between November

2012 and May 2014 to identify major sources of fine sediment particles and calculate their contributions. Sieved (20 µm) runoff samples were measured for turbidity, organic matter, oil and grease, elemental composition, and molecular organic markers. The contribution of fine sediment particles from major sources such as road hillside soil, abrasive sand, pavement wear, and vegetation debris was calculated using a chemical mass balance model. The most predominant source was surface soil from road hillside that contributed 20 to 70% (43 ± 9%) of fine sediment particles. It is interesting to note that asphalt pavement wear is the second most abundant source, which accounted for 12 to 53% (31 ± 8%) of fine sediment particles. The contribution of abrasive sand (Washoe sand) ranged between 7 and 23% (16 ± 4%). Turbidity peaked (over 1,000 NTU) during the first flush and declined exponentially. In the early phase runoff, turbidity was above 200 NTU, which is problematic for discharges to infiltration systems such as trenches, ponds, vaults, and porous pavement in the Lake Tahoe Basin. These results indicate that the first 30 to 50% (by volume) of runoff needs to be treated using treatment BMPs and the later phase of runoff (at least 50%, by volume) can be rerouted to downstream locations such as infiltration systems and pervious dispersion areas. This research supports that size and treatment capacity of treatment BMPs may be reduced to save geospatial footprint and cost.

**WP203 The Effect of Stormwater Outfall Relocation on Near Surf Zone Bacterial Exceedances in Myrtle Beach and North Myrtle Beach**

*M. Carson, University of South Carolina / Environmental Health Sciences; B. Rabon, D. Graves, South Carolina Department of Health and Environmental Control / Bureau of Water; G.I. Scott, University of South Carolina / Department of Environmental Health Sciences*

Ocean water quality monitoring informs the public of waterborne pathogen-induced illness risk. This monitoring allows for informed decisions to be made on ocean water contact recreation and public health, throughout the year. The Greater Myrtle Beach Grand Strand Region, including Myrtle Beach (MB) and North Myrtle Beach (NMB), has extensive bacterial pollution ocean monitoring. In 2002, routine monitoring was implemented, in South Carolina, as part of the BEACH Act. This program involves weekly ocean water quality monitoring at NMB and MB stations during beach season. High Enterococcus bacteria levels in ocean water, have been linked to an increased risk in human illness. Beach monitoring and issuance of timely advisories are key in the reduction of public health risk, related to ocean water exposure. Recent MB and NMB monitoring data indicated frequent exceedances of Enterococcus water quality standards with primary pollution sources emanating from nearshore storm water discharges. Thus, to reduce near shore bacteria loads, storm water outfall discharges were relocated offshore 1,000+ feet. Our study's goal was to determine if outfall relocation from on the beach to more than 1,000 feet out into the ocean had an effect on the number bacterial standard exceedances. To this end, we analyzed mean levels of both Enterococcus abundance and the percent exceedances for the Enterococcus Single Sample maximum Standard (104 cfu/100ml). Samples were compared before and after the outfalls were relocated. To discern differences alpha levels of 0.10-0.25 were used. Ten stations were examined between Myrtle Beach (N=5; WAC-018-WAC-024) and North Myrtle Beach. (N=5; WAC-005A-WAC-009).After outfall relocation, both MB and NMB areas demonstrated significant differences in Enterococcus levels. Generally, most examined outfalls had reduced levels of microbial pollution. The overall Enterococcus exceedances and average number of Enterococcusbacteria were reduced after outfall relocation. Taken together, our results suggest that ocean outfall implementation in Myrtle Beach and North Myrtle Beach, and the movement of storm water to further offshore locations could successfully decrease nearshore surf zone high bacteria incidences. Minimizing human contact with high-level bacteria pollution is essential for creating a safe, recreational environment for people to use and enjoy the ocean and beaches.

**WP204 A 30 Year Trend Assessment of Fecal Indicator Bacteria (*Escherichia coli* and Enterococci) Along the Shoreline of Santa Monica Bay, California**

*C. Enyart, J. Dorsey, Loyola Marymount University; K. Johnston, The Bay Foundation; D. Berube, Loyola Marymount University*

Santa Monica Bay and its vast beaches are important Los Angeles icons, while also providing significant ecosystem services to over millions of recreational visitors annually. Contaminated runoff from numerous watersheds surrounding the Bay, especially the 87% urbanized Ballona Creek Watershed, have historically resulted in poor water quality along areas of the Bay shoreline. Decades of monitoring for fecal indicator bacteria (FIB) along the Bay's shoreline has been associated with NPDES wastewater discharge and stormwater programs. Many projects have been implemented throughout the watersheds (e.g. sewer improvements, biofiltration systems, low-flow diversions (LFDs)) to lessen flows of runoff from contaminating surf zone recreational waters. Despite decades of monitoring, there has been no long-term assessment of trends in shoreline FIB, especially in response to implementation of projects to improve water quality. The goal of this study was to assemble 30 years of monitoring data (1988-2017) for *E. coli* and enterococci to assess trends along the entire shoreline of Santa Monica Bay. Raw data were acquired from various monitoring agencies to develop a data file that included 82 stations covering a set of about 150,000 observations (cfu or MPN/100 ml) for *E. coli*, and a similar number for enterococci. Data were analyzed by calculating rolling 30-day geometric means, and comparing means by geographic subdivision, between wet and dry weather, and over time. Resulting trends for both *E. coli* and enterococci were: 1) concentrations peaked around 2005 when many stations shifted to sampling points where runoff mixed directly with surf zone water; 2) after 2005, concentrations fell to present levels, especially at beaches where LFDs were implemented; 3) concentrations were extremely variable during the 2016-17 wet season; 4) the north and central areas of the Bay, impacted by runoff from the Ballona Creek and Malibu Creek Watersheds, had greater concentrations relative to the south area; and 5) dry weather concentrations were less than the TMDL standard of 35 MPN/100 ml, but did exceed this limit occasionally during wet weather in the central bay. Implementation of LFDs and other best management practices to restrict polluted runoff from flowing into the surf zones of the Bay's beaches most likely improved water quality throughout the Bay.

**WP205 Is dust a major transporter of synthetic pyrethroids in residential areas?**

*V.J. Pettigrove, The University of Melbourne / Sciences; D. MacMahon, CAPIM The University of Melbourne / Biosciences*

The synthetic pyrethroids (SPs) bifenthrin and permethrin have been detected in sediments of urban waterbodies throughout Melbourne, Australia and are often at concentrations that are toxic to fish and macroinvertebrates. Highest concentrations of these pesticides are often present in sediments from waterbodies near new residential developments where SPs are applied to houses to prevent termites infestation. Although these developments have swales, wetlands and other devices to intercept and treat urban stormwater, SPs are often present downstream of these treatment systems. A study was conducted to determine what were the major causes of SPs pollution from termite treatments, to understand how SPs enter local waterbodies and what can be done to reduce SP pollution from these activities. The termites can only access buildings through subsurface soils, so treatments involve creating a physical or chemical barrier around buildings. Chemical treatments are the preferred option on almost all houses. These include installing sheets impregnated with a SP, the use of an irrigation system primed with SPs and directly spraying the SPs around the perimeter of the building. To assess the contribution of surface runoff to pollution, 48 houses from 4 housing estates were monitoried for SPs in soil collected from within 50 cm of each building and from near the footpath where is was evidence of water runoff. Samples were collected at the start of construction, once construction was completed and the house was occupied and 6 months afterwards when the garden was usually more established. Soils were also collected from street gutters upstream and downstream of houses. Dust was collected from power boxes (~1.5m high X 2 X 3m) and other elevated structures. Bifenthrin, permethrin and occasionally cypermethrin were present in soils near buildings after SPs had been applied. Usually a small fraction of SPs was present in soils near the footpath where there were signs of surface runoff. Soils present in gutters, including those upstream of houses, also contained SPs. Dust also contained elevated concentrations of SPs. A strong correlation exists between SP concentrations in dust and the number of houses within a 50m radius of the site. Dust collected from the surface of power boxes more than 5km away from new housing estates also was contaminated with SPs, suggesting that dust can transport these substances long distances.

**WP206 Assessing Stormwater Impacts and Mitigation Measures in California's Areas of Special Biological Significance**

*K.C. Schiff, Southern California Coastal Water Res.*

The coastal environment of California is home to diverse and abundant marine life comprising some of the richest aquatic habitats on earth. In order to protect this valuable resource, the State has created 34 marine water quality protected areas termed Areas of Biological Significance (ASBS). Despite the prohibition against waste discharge and the mandate to maintain "natural water quality" in these marine water quality protected areas, nearly 1,700 stormwater outfalls exist in ASBS statewide. This project covers a five-year period tackling three critical management knowledge gaps. First, based on over 110 wet weather site-events at reference sites, natural water quality was translated from a narrative water quality standard to receiving water numerical thresholds. Second, based on more than 140 wet weather site-events, receiving water samples were collected near stormwater discharges into ASBS. The frequency, extent, and magnitude of stormwater impacts in these water quality protected areas was assessed using the reference-based numerical thresholds. All 34 ASBS statewide observed some level of impact, not necessarily co-varying with rainfall quantity, intensity, antecedent dry period or land use. Finally, more than $34 million (US) in State Water Bond grants was spent on stormwater infrastructure improvements and best management practices (BMPs). Monitoring of these BMPs was used to assess the effectiveness and efficiency of stormwater treatment and improvements to discharges into the State's water quality protected areas.

## Selenium Ecotoxicology and Management Going on 40: What Do We Know and How Can We Apply It?

**WP207 Toxicity of Aqueous L-Selenomethionine Exposure to Early Life-Stages of Zebrafish**

*A.K. Gerhart, University of Saskatchewan / Toxicology Centre; D.M. Janz, University of Saskatchewan / Veterinary Biomedical Sciences*

Selenium (Se) is an important micronutrient involved in numerous metabolic functions in vertebrates. However, its narrow range between essentiality and toxicity has generated concern due to elevated environmental concentrations. Numerous studies have shown that oviparous vertebrates, fishes in particular, are highly susceptible to elevated dietary selenium concentrations during early development due to maternal transfer. Exposure to seleno-L-methionine (SeMet), the predominant form of Se in the diet, during early life stages can induce teratogenic effects such as spinal deformities (lordosis, kyphosis, and scoliosis), edema, and fin and craniofacial malformations, as well as increase the incidence of mortality in developing fish larvae. Previous studies have largely consisted of dietary exposures, yolk microinjections, and aqueous exposures with juvenile or adult fish, with more recent work using aqueous embryo exposures. However, research comparing the effects of yolk microinjections to aqueous embryo exposures has yet to be conducted. Using *Danio rerio* as a model test organism, we sought to evaluate the effects of elevated SeMet, in solution, on developing embryos exposed during early life stages. Newly fertilized zebrafish embryos between 1– 3 hours post fertilization were aqueously exposed to graded concentrations of Se (5, 25, and 125 µg/L, ten replicates/treatment, 20 larvae/replicate) as SeMet for 5 days. Survival, hatchability, time to hatch, as well as the frequency and severity of deformities (total and type) were quantified. Whole-body Se concentrations were also determined in swim-up fry following the 5-day exposures. Although experiments are still ongoing, preliminary research suggests that exposure to SeMet, in solution, produces a dose-dependent increase in both the incidence and severity of deformities in developing zebrafish larvae. Through comparisons to other methods of exposure, this research has the potential to determine whether aqueous exposures represent a valid replacement for other labortaory methods of Se exposure such as spiked diets or yolk microinjections.

**WP208 Relative Sensitivity of Sculpin to Environmental Selenium**

*S. Covington, Formation Environmental; M.C. Lewis, Formation Environmental LLC; A. Prouty, JR Simplot*

In 2006, the J.R. Simplot Company began working toward the development of a site-specific selenium criterion (SSSC) for streams near one of its southeast Idaho mines. Relative sensitivity of resident fish is an important factor in developing the SSSC. Fish species present at the site include Brown Trout (BT), Yellowstone Cutthroat Trout (YCT) and Sculpin (primarily Pauite sculpin [*Cottus beldingi*], with many fewer mottled sculpin [*C. bairdi*]). Site-specific toxicity testing showed that BT were more sensitive to developmental toxicity from selenium than YCT, and are likely the most sensitive aquatic species present. Whole-body selenium EC10s (survival and development) were 13.5, and 14.5 mg/kg dw for BT and YCT, respectively. Sculpin were not included in toxicity testing, but data on population density (i.e., individuals per unit area), abundance within age classes, and whole-body selenium concentrations were available for an 11-year period, and were used to assess the relative selenium sensitivity compared to the trout species. Whole-body selenium concentrations in sculpin exceeded those in trout, with average concentrations ranging up to about 27 mg/kg dw in selenium-affected sampling locations. Sculpin population (density and age class frequency) was not affected in a dose-dependent manner relative to selenium in surface water or whole-body tissues. The relative proportion of populations represented by the youngest, and most likely affected, age class (years 1 and 2, length < 60 mm) was unrelated to whole-body selenium concentration from 2 to 27 mg/kg dw. Age class 3 and 4 (length 60-90 mm) sculpin represent fish that have survived to reproductive age. While abundance of this age class fluctuated year-to-year at both background and selenium-affected sampling locations, the mean abundance in 2013 was similar to 2006, despite increasing selenium concentration in whole-body and surface water. The variability was not correlated with whole-body selenium concentrations or selenium concentrations in surface water at the most selenium-impacted locations. The population and age-class endpoints are not directly comparable to developmental toxicity endpoints, but the apparent lack of effect of high whole-body selenium concentrations on key population metrics indicate that sculpin may be more tolerant of selenium toxicity than YCT.

**WP209 The Mystery of the Naturally Selenized Fish**

*L. Ashby, University of Ottawa / Earth Sciences; K. Hille, M. Bromstad, S. Earle, K. Salzsauler, Golder Associates, Ltd.; A. deBruyn, Golder Associates, Ltd. / Environmental Science*

Baseline studies in a pristine oligotrophic lake in northern British Columbia found that resident rainbow trout (*Oncorhynchus mykiss*) had tissue selenium concentrations exceeding health-based consumption guidelines, despite aqueous selenium concentrations less than 1 µg/L. We conducted a study to identify the source of selenium to these fish. Selenium speciation analysis of sediment cores and dialysis array ('peeper') water samples across the sediment-water interface indicated that lake sediments were a net sink, not source, of selenium. Organoselenides were present in all tested surface sediment samples. Gut content and stable isotope analysis revealed that rainbow trout (the only fish species present in the lake) were broadly omnivorous, feeding predominantly on planktonic, benthic, and littoral invertebrates. Selenium analysis of potential prey in several areas of the lake identified few taxa with sufficiently high concentrations to explain concentrations in rainbow trout. Most invertebrates, including those that predominated in fish guts, had tissue selenium concentrations less than 5 mg/kg dw, whereas rainbow trout had muscle selenium concentrations ranging from 7 to 28 mg/kg dw and egg selenium concentrations up to 48 mg/kg dw. Benthic chironomids and littoral caddisflies had distinctly higher concentrations than other taxa, up to 17 mg/kg dw. However, approximately 20% of rainbow trout captured had tissue selenium concentrations too high to be explained by the invertebrate prey sampled. A possible explanation is cannibalism. One fish gut contained a juvenile rainbow trout and the highest-selenium fish also had elevated $\delta^{15}N$, consistent with feeding on juveniles. Our data suggest that the high baseline fish tissue selenium concentrations may have resulted from preferential feeding of juveniles on high-selenium invertebrate prey and cannibalistic feeding of adult rainbow trout on those juveniles.

**WP210 Sensitivity of Arctic Grayling (*Thymallus arcticus*) Larvae Exposed to Selenium via Maternal Transfer**

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Selenium toxicity to fish is primarily manifested via maternal transfer to the eggs, which may result in adverse effects on larval survival and development. In this study, gametes were collected from Arctic grayling (*Thymallus arcticus*) collected from Alaska streams with a range of selenium exposure concentrations. Eggs were fertilized and reared in the laboratory from hatch through post-swimup. Larvae were assessed for survival, length, weight, and deformities (skeletal, craniofacial, finfold) and edema based on a graduated severity index (GSI). No adverse effect relationships were observed between larval endpoints evaluated and parent females' egg, muscle, or whole-body selenium concentrations. Therefore, selenium toxicity thresholds (10% effect concentrations [EC10s]) were defined as the maximum measured selenium concentrations: >33.9, >17.6, and >19.7 µg/g dry weight for eggs, muscle, and whole-body tissue, respectively.

**WP211 Factors influencing selenium uptake by periphyton in boreal lake ecosystems**

*M.D. Oldach, University of Saskatchewan / Toxicology Centre / Veterinary Biomedical Sciences; D.M. Janz, University of Saskatchewan / Veterinary Biomedical Sciences*

Selenium is an essential trace element with a narrow margin between essentiality and toxicity in many organisms. Selenium is a contaminant of concern in the boreal forest region of North America because certain industrial practices release selenium into cold-water aquatic environments. Selenium is rapidly assimilated into food webs by primary producers, including algae, bacteria, and fungi, and transferred to higher trophic levels through dietary pathways. There are significant site-specific differences in the uptake of selenium into food webs by primary producers, which can have implications for higher trophic levels. Therefore, the objective of this study is to diminish this uncertainty through quantifying selenium uptake at the base of the food web in representative boreal lake ecosystems. This will be achieved by 1) investigating if there are concentration-dependent differences in periphyton enrichment functions of selenium, and 2) determining if water chemistry plays a role in selenium uptake by periphyton. Textured glass plates were deployed in 5 lakes with varying levels of dissolved organic carbon and total phosphorous at the IISD-ELA (International Institute for Sustainable Development-Experimental Lakes Area). After a growth period of 6-8 weeks, plates were exposed to environmentally relevant concentrations of selenium (control [0.1-0.2], 0.5, 1, 2, 4 µg Se/L) as selenite, and allowed to incubate in a static renewal system under ambient conditions for 8 days. Aqueous dissolved and total selenium concentrations, selenium accumulation in periphyton tissues, and periphyton community structure were quantified. The results of this research will provide insight on the biodynamics of selenium assimilation at the base of boreal lake food webs, which can potentially inform ecological risk assessment in cold freshwater ecosystems in North America.

**WP213 Selenate versus selenite – uptake into algae and trophic-transfer to *Daphnia magna***

*C. Rickwood, M. King, E. Suominen, G. Prabhakar, Natural Resources Canada*

The uptake of Selenium from water to primary producers has generally been accepted as the most important stage in the toxicity of Se, due to accumulation and conversion to the organic form Se-methionine. The objective of this study was to determine whether speciation of Se in water is important in determining trophic-transfer and toxicity. This presentation will provide a comparison between Selenate and Selenite exposed algae and the effects of dietary exposure to the cladoceran *Daphnia magna* over two-generations. An algal diet, consisting of both *Chlorella kessleri* and *Pseudokirchneriella subcapitata* was spiked in a dose-dependent manner with Selenite and Selenate over a 7-day growth period resulting in a dietary-Se exposure of 5, 20 and 80 mg/kg Se (Nominal Concentrations). Selenium speciation in growth media during 7-days and total Se in algae was measured to determine dietary exposure concentrations. First generation *D. magna* were fed Se-spiked algae over a 21-day period to assess survival and reproduction. Offspring were collected and either exposed to dietary-Se or a control diet for a further 21-days. Tissue concentrations of both 1st and 2nd generation *D. magna* were assessed as well as visual inspection of the daphnids for any potential deformities. A comparison of algal uptake, speciation in growth media, daphnid reproduction, survival and tissue Se concentrations, including trophic-transfer factors, will be presented.

**WP214 Development of a Draft Selenium Aquatic-Dependent Wildlife Ambient Water Quality Criterion for the State of California**

*A. Jarvis, J. McLaughlin, B. Schnitker, J.R. Beaman, K. Gallagher, USEPA / Office of Water; D. Oros, USEPA Region 9*

Selenium is found in aquatic ecosystems in California. At low concentrations, selenium is an essential nutrient. However, selenium concentrations slightly above beneficial levels can be harmful to wildlife (Ohlendorf 2003). The bioaccumulation of selenium through the aquatic food web can affect consumers and predatory organisms. Along with other oviparous vertebrates, such as many fish, birds appear to be particularly sensitive to selenium exposure. Malformations and reproductive impairments have been observed at environmentally relevant concentrations in aquatic-dependent bird species (Ohlendorf et al. 1986; Hoffman et al. 1988). USEPA has initiated development of a selenium aquatic-dependent wildlife criterion focused on California ecosystems using latest scientific literature and building upon the approach developed in the national 2016 USEPA Aquatic Life Selenium Criterion.

**WP215 Preliminary Risk Assessment for Avian Exposure to Selenium at Lake Koocanusa, Montana**

*J.P. Skorupa, K.J. Nelson, US Fish and Wildlife Service / Environmental Contaminants Program*

Lake Koocanusa is a trans-boundary reservoir in northwestern Montana and southeastern British Columbia, created in 1972 with the completion of Libby Dam on the Kootenai River. Coal mining operations in the Elk Valley watershed of British Columbia discharge selenium (Se) into the Elk River, a major tributary to Lake Koocanusa. Annual Se loads entering Lake Koocanusa have increased from 2,600 kg in 1992 to over 13,000 kg in 2012, more than a fivefold increase. The Montana Department of Environmental Quality has listed Lake Koocanusa as impaired for Se under sec. 303(d) of the Clean Water Act, and fish tissues often exceed risk benchmarks for fish. Aquatic ecosystems that exceed Se toxicity benchmarks for fish tissue also present the potential for toxicological risk to aquatic-dependent birds; however, no systematic surveys of avian breeding activity or sampling of avian tissues had been conducted at Lake Koocanusa in Montana. During the breeding season of 2016 a multi-agency team of biologists conducted a reconnaissance survey of nesting birds at Lake Koocanusa and collected a small set of shorebird eggs for Se analysis. Previous research on Se in shorebird eggs along the Elk River showed a strong declining gradient of exposure as a function of distance from the coal mines, and our results for shorebirds at Lake Koocanusa matched-up with that gradient. We also conducted a Monte Carlo analysis of pre-existing egg data from the Elk River watershed to estimate a site-specific toxicity benchmark for shorebird eggs that revealed our results were well below that estimated toxicity benchmark. Swallows are the second most abundant type of nesting bird at Lake Koocanusa. In the absence of egg samples for this taxonomic group, we conducted an indirect assessment based on an array of other Se contaminated sites with exposure information for co-occurring shorebirds and swallows that allowed a working hypothesis to be generated for expected Se exposure in swallow eggs at Lake Koocanusa. Combined with Se response data for swallows from the literature, our working hypothesis suggests that collecting Se exposure data for swallow eggs at Lake Koocanusa is a warranted follow-up avian research priority. However, both the direct assessment for shorebirds and the indirect assessment for swallows suggest that Se exposure of fish, not birds, will be the most vulnerable biological endpoint to focus on for setting a site-specific water quality standard.

**WP216 Developing Site-Specific Selenium Criteria: Challenges and Lessons Learned**

*S. Covington, Formation Environmental; A. Prouty, JR Simplot*

In 2006, the J.R. Simplot Company began working toward the development of a site-specific selenium criterion (SSSC) for streams near one of its southeast Idaho mines. At that time, the selenium standard in Idaho was a water value of 5 ug/L. Developing information at that time from the scientific and regulatory community suggested that a new selenium criterion should be based on the response of organisms relative to their tissue concentrations. The consensus indicated that selenium is bioaccumulated through dietary intake and effects are manifested in the developing young, thus exposure is primarily dietary, not water borne, and tissues are the best measure of exposure. In 2016, the USEPA released its National Selenium Criterion for water based on a four-part criterion that included thresholds for egg/ovary, whole body/muscle, and water elements. The following year, Simplot put forth a proposal to Idaho DEQ for a SSSC based on an egg effect threshold for brown trout. Simplot's proposal used the USEPA criteria format to derive criteria for egg/ovary and whole-body fish tissues, and water using site-specific data. Idaho DEQ vetted the SSSC initially through a workgroup convened to help guide the process, and later through its formal rulemaking process which included public comments and reviews from individuals and state and federal agencies. In between these two processes, additional vetting was conducted by USEPA and USFWS. Following multiple comment periods, meetings, and reviews, Idaho DEQ adopted the SSSC with revisions, and submitted the rulemaking to the Idaho legislature for adoption. This presentation will provide some unique perspectives on the SSSC development process, derivation of the proposed criterion as well as the proposal and rulemaking process. Through navigating these processes, Simplot has compiled some "lessons learned" that may be useful to other entities that choose to undertake the SSSC process.

**WP217 Large-scale selenium remediation by ecosystem manipulation: N-P fertilization of a mine-pit lake stimulates anaerobic Se reduction**

*A. Luek, University of Lethbridge / Department of Biological Sciences; D.J. Rowan, Environmental Science Branch, Canadian Nuclear Laboratories; J.B. Rasmussen, University of Lethbridge / Department of Biological Science*

Open-pit coal mining can introduce selenium (Se) rich leachate to watersheds receiving surface runoff from the surrounding mined landscape. The associated watersheds often require large-scale, long-term treatment to mitigate Se toxicity in order to protect the health of aquatic life downstream. End-pit lakes are a standard mine-closure method for surface coal mining. Hence a vast number of these waterbodies remain in the landscape long after mining activities cease. End-pit lakes continuously accumulate Se from the surrounding watershed. High water residence times in the pits require new methods for Se management and remediation, as current practices are predominantly based on flowing water. To reduce Se in an end-pit lake, we applied the principles of anaerobic bacterial bioreactors, previously successful in small scale Se mitigation, to a whole pit lake ecosystem. The fertilization of a pit lake with N and P increased primary production, which established a meromictic anoxic layer, when senescing algae sank and decomposed. The increase of this anoxic layer enhanced habitat for locally present, anoxic, Se and sulfur respiring bacterial communities. Anaerobic microbial reduction processes then reduced Se-oxyanions by ten-fold within two years post fertilization to below water-quality guideline values. This successful application of a small scale bioreactor principle to a whole lake ecosystem allows for a low maintenance treatment of large quantities of mine drainage water, suitable to be utilized even in remote environments. A review of more than 50 coal mining end-pit lakes in Alberta, Canada showed approximately 60% of the lakes to have physical and chemical environments suitable for the here described treatment method. With high numbers of Se contaminated, new and legacy end-pit mine lakes globally, direct lake ecosystem manipulation could provide an inexpensive method for the management and reduction of a persistent aquatic contaminant in situ.

## Exposure to Crude Oils: Linking Laboratory Endpoints to Field Observations

**WP218 Toxicological assessment of a novel unimolecular micelle nanoparticle for oil dispersant using Fathead minnow, embryos and juveniles**

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Chemical dispersants have been used as a tool to remediate oil spills. However, traditional chemical dispersants depend on their concentration to form micelles to disperse oil, also called their Critical Micellar Concentration (CMC). Furthermore, there is an increasing demand to use environmentally friendly oil dispersants with high ability to clean up oil without toxicity. The aim of this project is to analyze the effects of a novel unimolecular micelle, engineered based on a silica nano-sized core with amphiphilic branches, conferring to the nanoparticle (NP) the characteristics of a traditional oil dispersant but without the need of achieving a CMC. Furthermore, as the NP can encapsulate better the organic compounds, their toxicity could be decreased. Fathead minnow embryos (~24hpf) and juveniles (10dph) were exposed to NPs resuspended in two different media: one to test the toxicity of NP alone, and a second to test toxicity of an oil spill simulation using the oil water accommodated fraction (WAF) mixed with NPs at concentrations of 2 mg/L, 10 mg/L, and 50 mg/L. WAF was made after weathering for one week crude oil with 20% Hank's solution (27.348 mM NaCl, 1.073 mM KCl, 0.0493 mM $Na_2HPO_4$, 0.0881 mM $KH_2PO_4$, 0.2523 mM $CaCl_2$, 0.196 mM $MgSO_4$, 40.833 mM $NaHCO_3$ ) to make a final concentration of 1 ml/L. NPs were resuspended with sonication into Hank's (20%) and then used as is or mixed with WAF 24hrs prior use. Exposures treatments were done in quadruplicate, with daily 50% solution changes for a total of 96 h. Mortality was monitored and removed on a daily basis. Toxicological endpoints assessed were embryo malformation, embryo heartbeat and in both stages gene expression of *cyp1A*, a gene that is transcriptionally activated after exposure to polyaromatic hydrocarbon compounds in crude oil. Preliminary results among embryos showed that the treatment with WAF and 2 mg/L of NP increased mortality. Furthermore, a significant decrease in heartbeat was observed with 2 mg/L NP treatment alone, WAF alone and WAF with 10 mg/L of NP (p< 0.001). Toxicity related effects were seen only on embryos, highlighting the sensitivity of this life stage. Experiments to measure internal bioavailability of oil in terms of gene expression are in progress. These preliminary results show that low concentration of NP alone and with WAF have some effects to fathead minnow embryos.

**WP219 Reproduction defects and tumorigenesis risk in *Caenorhabditis elegans* following exposure to crude oil-dispersant mixture**

*X. Pan, T. Thornburg, East Carolina University / Biology; Y. Zhang, East Carolina University*

The crude oil mixed with dispersant used in cleanup efforts represent a well-defined mixture entity that warrants investigations of its potential toxicity and health risks. Previously we have reported that the exposure to crude oil and the dispersant mixture induced reproduction defects such as decreased brood size, increased germ cell apoptosis, and abnormal spermatogenesis in *Caenorhabditis elegans* (*C. elegans*). Here, we report our new findings on the crude oil-dispersant mixture induced reproduction toxicity. Long-term exposure of *C. elegans* male strains to oil-dispersant mixture results in abnormal spermatid morphology and less motility of mature sperms. Furthermore, we utilized puf-8; lip-1 tumor sensitive strain to test the cell fate of immature sperms induced by Dis-Oil mixture treatment. Our study suggests that oil-dispersant mixture induce tumorigenesis in puf-8; lip-1 strain of *C. elegans* in a dose and temporal dependent manner. The related gene expression and microRNA-mediated mechanism will also be discussed.

**WP220 Monitoring of PAHs in shellfish after oil spill from the Sanchi tanker; comparison of polluted conditions with other spill accident using ChemTHEATRE**

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Until now, there have been countlessly the monitoring data for chemical contaminants. However, most of them perhaps ineffectively utilized, because their data were sometimes reported only a part without supplemented data and difficult to find the required data effectively. ChemTHEATRE (http://chem-theatre.com/) is the platform to deposit and visualize monitoring data of environmental contaminants. ChemTHEATRE describes that this database will ensure traceability of chemicals and help to simulate the environmental behavior and fate, or assess the risk. On January 2018, the *Sanchi* tanker sank off the coast of Okinawa, Japan. Most of condensate for 136,000 *ton* put in the tanker spilled and could diffuse around Japan. In addition, the heavy oil perhaps spilled from Sanchi had been drifted at coastal areas of Amami and Takara Islands, Japan from February 2018. In this study, we collected biological samples as shellfish at some locations of both islands on March 2018, and investigated PAH concentrations these. In this spill accident, it is difficult to distinguish whether the pollution is serious or not. Therefore, we also will try to compare the polluted condition with PAHs in aquatic organisms between Sanchi accident and other spill recorded in ChemTHEATRE, for example, oil spill off the coast of Guimaras Island, Philippines 2006.

**WP221 The BOREAL Project: Preliminary results from a simulated spill of diluted bitumen in boreal lake enclosures**

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In recent years, a number of reports by the US National Academies, the Royal Society of Canada, and United States Geological Survey and among others, have highlighted the numerous knowledge gaps that currently exist in relation to the weathering, fate, behaviour, and environmental effects of diluted bitumen (dilbit) after a potential spill into aquatic systems. These reports emphasized the need for field research to address these gaps, with special need for data on freshwater systems. After a successful pilot study in 2017, the BOREAL project (Boreal lake Oil Release Experiment by Additions to Limnocorrals) set up to address some of these research needs in the summer of 2018 with a first-ever, large-scale, field simulation of a dilbit spill in a boreal freshwater system. Nine 10-m diameter, 157 m3, enclosures were set up in lake 260 at the International Institute for Sustainable Development's Experimental Lakes Area (IISD-ELA). A regression design with 2 controls and 7 oil treatments was used to assess the weathering, fate, behaviour, environmental effects and bioaccumulation potential of dilbit and its components. Nominal treatments ranged from 1.6 to 160 L of dilbit, resulting in oil:water ratios ranging between 1:100,000 to 1:1000. This talk will present a summary of initial findings from all components of the project (oil fate and behaviour and ecotoxicological effects) paying special attention to semi-quantitative data on the general behaviour of the oil (e.g. floating vs sinking) throughout the 80 days of the study.

**WP222 Understanding the effects of diluted bitumen in fresh waters through experimental oil spills: Preliminary findings on microbial communities**

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The overall goal of the BOREAL (Boreal lake Oil Release Experiment by Additions to Limnocorrals) study is to advance our understanding of the fate and effects of diluted bitumen (dilbit) in freshwater ecosystems in Canada. We have conducted a series of aquatic mesocosm experiments at the IISD-Experimental Lakes Area, a unique freshwater research facility situated in north-western Ontario. In 2017, we performed a pilot study using three land-based 2-m diameter microcosms, and in 2018 an in-lake study using nine 10-m diameter limnocorrals was executed. Two of the questions we aimed to answer were: (i) what are the effects of dilbit on the structure and function of microbial communities?; and (ii) what role do microbial communities play in the fate of dilbit in freshwater ecosystems? To address the first question, we used a multifaceted approach, from genomics to microscopy, to examine changes in the biomass, species composition, and diversity of bacteria, viruses, and phytoplankton in response to a comprehensive range of dilbit spill scenarios (i.e., oil-to-water ratios from 1:100,000 to 1:000). We also quantified important ecosystem functions such as primary production and decomposition. To address the second question, we imaged oil-particle aggregates to elucidate the role of phytoplankton in oil sedimentation and employed transcriptomics to better understand the role of bacteria in oil degradation in freshwater ecosystems. In addition to advancing our understanding of dilbit fate and toxicity in fresh waters, we anticipate the results of the BOREAL study will inform evidence-based policy decisions and sound environmental monitoring programs.

**WP223 The effects of diluted bitumen with and without dispersants on Pacific marine benthic organisms**

*C.J. Kennedy, K. Rhodenizer, Simon Fraser University / Biological Sciences*

The higher-level effects of diluted bitumen (dilbit), dispersed dilbit, and dispersant (Corexit 9500A) exposure to marine benthic species was examined. Water accomodated fractions of dilbit (WAF) or chemically enhanced WAF (CEWAF; dispersed WAF) were generated using the CROSERF method. The susceptibility of different groups of marine benthic organisms in acute toxicity tests varied from being relatively non toxic at 100% WAF to lethal at less than 5% dilutions. CEWAF was always more toxic to all species compared to WAF alone, however the magnitude of CEWAFs higher toxicity was not the same in all species. Susceptibility trends for WAF were Kelp (*Macrocystis pyrifera)*> Echinoderm (*Strongylocentrotus purpuratus)*> mysid (*Mysidopsis bahia)*= amphipod (*Eohaustarius estuarius*) = polychaete (*Alitta virens*) > spot prawn (*Pandalus platyceros*) = tidepool sculpin (*Oligocottus maculosus*). LC50 values for the latter 2 organisms could not be determined for WAF. Several performance abilities showed significant alterations in organisms upon exposure. WAF/CEWAF at any dilution did not affect the proportion of prawns that molted. Prawns, amphipods and polychaetes-responded variably to the presence of low concentrations of dilbit; some organisms were either not able to detect, or to respond to dilbit suggesting a greater risk of exposure (particularly if attracted). Most organisms detected and avoided CEWAF and Corexit. These studies also show that behaviours including olfactory ability in prawns, and burrowing behaviour in amphipods and polychates is affected by exposure to WAF, CEWAF, and Corexit. This toxicity data provides useful information for assessing the risks of diluted bitumen, dispersed bitumen and dispersants to benthic marine organisms.

**WP224 The effects of non-dispersed and dispersed diluted bitumen on the tidepool sculpin (*Oligocottus maculosus*)**

*S. Modares, E. Ardeshirlarijani, C.J. Kennedy, Simon Fraser University / Department of Biological Sciences*

Canada is the sixth largest oil producing country in the world, with an average output of 197,000 $m^3$/d of bitumen mostly from oil sands in northern Alberta. Canadian pipeline companies have proposed a number of major new transmission pipelines from this area and new marine terminal development for tanker export of bitumen to overseas markets increasing the potential risk of a marine spill. Few studies exist on the potential lethal and sublehthal toxicity of dilbit to any marine organism. Key information from toxicological studies of crude oil and its components such as BTEX and PAH may be applied to dilbit, however, generalizations on risk cannot be made with any certainty. Water accomodated fractions of dilbit (WAF) or chemically enhanced WAF (CEWAF; WAF dispersed with Corexit 9500A) were generated using the CROSERF method. Young adult tidepool sculpin (*Oligocottus maculosus*) were exposed to varying dilutions of WAF, CEWAF or concentrations of Corexit 9500A for >12 weeks. Acute toxicity and sublethal effects were examined at various levels of biological organization from the biochemistry to behaviour. WAF was not acutely toxic to sculpins, however both CEWAF and Corexit exposures resulted in mortality and the generation of LC50 data. Exposure to WAF, CEWAF and Corexit caused a short-term stress response and an increase in oxygen consumption. Exposure to CEWAF and Corexit reduced growth by the end of the exposure period. Sculpins avoided only the high concentration of WAF, but avoided all concentrations of CEWAF and Corexit. Exposure to WAF, CEWAF and Corexit altered several behaviours including those associated with swimming and feeding. The studies here provide comprehensive data on the potential impacts of dilbit and dispersants on teleosts and will aid in the development of risk assessment plans for managing marine organisms in the event of potential dilbit spills in this and other coastal ecosystems.

**WP225 Mixture toxicity of true boiling point distilled fractions of Iranian Heavy Crude oil**

*S. LEE, Korea University; J. Kwon, Korea University / Division of Environmental Science and Ecological Engineering*

Marine oil spills are some of the most serious environmental problems worldwide as the oil-spill residues cause adverse ecological effects. Crude oil is a complex mixture composed of many components including hydrophobic hydrocarbons that are known to accumulate in organisms and to cause toxicity. It is acknowledged that the short-term toxicity of aromatic hydrocarbon mixtures, which are thought to be the main toxic components in oil residues, is explained by the concentration-addition model. However, only limited number of chemicals from oil mixtures was studied to evaluate whether the concentration-addition model is a good mixture toxicity model for short-term ecotoxicological endpoints. In this study, the luminescence inhibition of *Aliivibrio fischeri* was evaluated using true boiling point (TBP) distilled fractions of Iranian Heavy Crude oil (IHC) and mixtures composed of those fractions. Thirty-one TBP distilled fractions of IHC were obtained at TBP from 70 to 400 ?. The water accommodated fractions of 31 distilled fractions and 50 mixtures composed of 3 randomly selected distilled fractions were prepared, and the luminescence inhibition of *A. fischeri* by them was evaluated. From dose-response curves of 31 distilled fractions, the toxicity of 50 random mixtures was predicted by the concentration-addition model and the response-addition model. There were no significant differences in results predicted by the two models, and the experimental results were well described by the concentration-addition model for most of mixtures. All experimental toxic units (TUs) of random mixtures were within the range of ±50% from the predicted TUs using the concentration-addition model and TUs of seven mixtures deviated more than ±30% from predicted, although the experimental TUs of random mixtures were mostly lower than predicted. The weighted sum of TU of 31 TBP distilled fraction was 3.47, which is close to the TU 4.31 of the whole IHC WAF. This indicates that there are no significant interactions between constituents of oil-mixtures in luminescence inhibition of *A. fischeri*, and it is expected that the concentration-addition model could be used for the prediction of luminescence inhibition, a widely used short-term ecotoxicity endpoint, by various mixtures of crude oils.

**WP226 Establishing baseline biomarker levels in commercially important demersal fishes**

*M. Gagnon, Curtin University / School of Molecular and Life Sciences*

The Browse Basin off Western Australia is an area of significant oil and gas exploration and extraction. Large extents of the Browse Basin are practically undisturbed due to the remoteness of the area where exploration and extraction takes place, with only a few small scale commercial fisheries operating in the area. In order to fully predict and manage the impacts of a hydrocarbon spill on fisheries resources, the capacity to detect the exposure to and effect of point sources of hydrocarbons is required. The objective of this study was to establish baseline data for commercially important demersal fishes for which effects of hydrocarbons could be evaluated in the event of an oil spill. More precisely, the study focussed on baseline physiological and biochemical markers of fish health which can establish recent exposure to petroleum hydrocarbons, and/or induced health effects following exposure. Complementary chemical quantification of petroleum hydrocarbons in water, sediments and fish flesh were conducted at all nine sampling sites. The commercially important species goldband snapper (*Pristipomoides multidens*) and red emperor (*Lutjanus sebae*) were targeted using commercial fish traps. Within a species, some inter-site differences were identified in biochemical marker levels which might be associated with different water temperatures, food supply or other site-specific conditions. These variations are not indicative of good or ill health status in fish but rather, represent baseline levels at each sampling locations to which future investigations can refer to. A chemical characterisation of the surface water, water column, bottom sediments and fish fillets was undertaken to complement physiological and biochemical assessments of fish. While PAHs were detected in all environmental compartments, the levels at which they were detected were considered as background environmental levels. Specifically, the chemical characterisation did not provide evidence of the presence of petroleum hydrocarbons at any of the study sites that would indicate anthropogenic or natural sources of hydrocarbons. Hence the levels of biomarkers reported here at each site are considered an appropriate baseline of non-exposed fish.

## Modeling and Data Analysis Tools to Predict the Fate and Transport of Pesticides in the Environment

### WP227 Developing a selection procedure for meteorological data used by regulatory AERMOD modeling of pesticide applications in California

*J. Tao, California Department of Pesticide Regulation*

We developed a procedure to select a set of 5–year meteorological data as the worst-case scenario data for regulatory modeling of pesticide uses. We analyzed the relationship between the 95$^{th}$ percentile maximum concentrations estimated by AERMOD and the percentages of low wind speed (LWS, 0.5 – 2 m/s) in the meteorological data used for the modeling. The results of statistical analysis showed that they were positively correlated within various distances to different types of emission sources. In addition, the LWS percentages of 1-year data at a station could be used to predict the LWS percentages of 5-year data at the same station. Based on these results, our procedure begins with the evaluation of 1-year data quality and LWS percentages for all the available stations in areas with high use of a concerned pesticide. Five – year meteorological data are then processed for the top 5 or top 20% of stations with the highest LWS percentages to perform AERMOD modeling. Finally, the air concentration estimates are compared to determine the worst-case scenario data. This procedure provided a strategic plan for the selection of meteorological data. We applied the procedure in a case study of sulfuryl fluoride structural fumigation of residential houses and determined that 5-year (2011 – 2015) data of station WBAN 93134 is the worst-case scenario meteorological data.

### WP228 Using SWAT Model to Assess Pesticide Runoff from Central California Coastal Watershed

*Y. Xie, California Department of Pesticide Regulation; X. Zhang, California Department of Pesticide Regulation / Environmental Monitoring; Y. Luo, California Department of Pesticide Regulation*

The California Department of Pesticide Regulation's Surface Water Protection Program (DPR/SWPP) relies on modeling tools for pesticide registration evaluation and post-use risk characterization. This study aims to develop a modeling approach that links pesticide use and the associated risks in aquatic ecosystems, or "use-based modeling." DPR's Pesticide Use Reporting (PUR) database, which reports agricultural uses at section level (1 × 1 mile) and urban uses at county level, is used to derive pesticide use information. The Soil and Water Assessment Tool (SWAT) is used to assess the temporal-spatial variation in pesticide runoff, loadings, and in-stream concentrations in the Central California Coastal Watershed. A whole range of components, including land use, soil property, slope, meteorology, pesticide application and drift, crop growth, water management, in-stream processes are considered to simulate various physical processes occurring in the watershed, and predict fate and transport of pesticides in the system. The modeling approach developed by the study will be used to identify hotspots and critical source areas of pesticide contaminates in surface waters, assess the efficiency of regulation over time and space, and evaluate the effectiveness of management practices in mitigating pesticides in the watershed.

### WP229 Predicting the Effects of Plant Growth Stage and Species on the Volatilization of Pesticides from Agricultural Fields after Application

*M.R. Taylor, University of Otago / Chemistry*

Pesticides are an important tool in modern crop management to ensure high quality and high yield food production. However, pesticide volatilization from agricultural fields can have a significant impact on nontarget, sensitive ecosystems, and can result in a loss of revenue for farmers. We developed and validated a model, based on multiphase partitioning, that describes pesticide volatilization loss from a planted agricultural field and predicts 24-h cumulative percentage volatilization ($CPV_{24h}$) losses. The model allows the user to adjust the chemical-physical properties of the pesticide, species-specific plant surface chemistry, plant size, soil type and climatic conditions. A general equation for plant-air ($K_{plant\text{-}air}$) partition coefficients was generated and implemented in the model to describe the plant-surface chemistry interactions with pesticides. The model was used to address questions about whether pesticides tended to volatilize more from plants or soil, and how plant species and growth stage affect the amount of pesticide lost to the atmosphere. Results are displayed on chemical space diagrams for sets of hypothetical $K_{plant\text{-}air}$, $K_{soil\text{-}air}$ and $K_{water\text{-}air}$ combinations under different scenarios. The $CPV_{24h}$ increased with increasing temperature, foliar intercept fraction and windspeed and with decreasing plant size. We found that pesticides tended to volatilize more from plants than soil. $CPV_{24h}$ was highly varied between plant growth stage but was less varied between plant species. The model was then compared to current screening techniques employed by the EU and the USEPA for the risk assessment of pesticides. Current screening techniques use vapor pressure as an indicator of the risk of pesticide exposure in the atmosphere. However, $CPV_{24h}$ had no correlation with vapor pressure as this does not account for the multiphase interaction that occur in the real environment. The current techniques tended to overestimate the volatilization potential of high-vapor pressure pesticides, which can lead to a number of pesticide undergoing unnecessary screening and testing. Our model is therefore a promising new screening tool for pesticide volatilization from an agricultural field.

### WP230 A data-driven approach for prediction of pesticide dissipation half-lives in plants

*F. Gao, S. Boyd, Michigan State University / Department of Plant, Soil and Microbial Sciences; E. Zhao, Beijing Academy of Agriculture and Forestry Sciences / Institute of Plant and Environment Protection*

Pesticides are widely used to protect crops against various harmful pests in modern agricultural production. However, residues of pesticides in plants such as fruits and vegetables can potentially cause harm to humans and animals that consumed the plants, and has been a great concern in risk assessment. The dissipation process of pesticides in plants is one of the key studies during pesticide risk assessment. The half-lives of pesticide are important for estimation of pesticide concentrations found in plants at harvest time. Since the dissipation of pesticides from plants are related to multiple factors such as pesticide properties, surrounding environments and plant types, the prediction of pesticide dissipation half-lives has been a challenge and thus are heavily relied on experiments. But the experiments on dissipation half-life studies are limited compared with the large amount of pesticide-plant combinations. Prediction models would be useful for assessing all possible pesticide−plant combinations based on the available data. Since the processes of dissipation are not fully understood, in this study, we applied a data-drive approach using artificial neural network models to predict the dissipation half-lives. The chemical structures of pesticides were regarded as molecule graphs and unique molecule features were generated based on the random walk over the graph. To test the reliability of our feature extraction methods, we predicted the octanol-water coefficient($k_{ow}$) of pesticides which should be only related to the structures. Then these pesticide features were combined with other features (i.e. plant types, compartment parts) to form new features and fed into an artificial neural network to predict the Log(Half-life). Despite that very few knowledge of surrounding environments was available, our model tried to make the predictions based on limited information. First, we removed irrelevant data points in current datasets and then collected 100 more data points published in recent three years. Secondly, we further analyzed the structures of pesticides in the datasets and discussed its relation to dissipation half-lives. Finally, with 70% data point as training, 10% as validation and 20% as test, our model achieved state of art prediction results with mean absolute error($MAE_{log}$) 0.60. The results indicated that our deep neural network models can be successfully used for the prediction of pesticide half-lives in plants.

**WP231 Identification of the most influential watershed characteristics for pyrethroid pollution in stream sediments in California**

*D. Wang, R. Budd, C. DeMars, California Department of Pesticide Regulation; B.M. Phillips, B.S. Anderson, University of California, Davis / Environmental Toxicology; N. Singhasemanon, K. Goh, California Department of Pesticide Regulation*

Pyrethroids are a group of insecticides under intensive investigation in California because of their high use and high toxicity to aquatic invertebrates. This group of pesticides tends to have a high affinity for soil and sediment particulate matter. Their fate and transport in the aquatic system are largely associated with the movement of sediments and appear to be greatly influenced by the hydrological, geomorphological and anthropogenic characteristics of the contributing watershed. This study develops a statistical assessment procedure to investigate the link between the watershed characteristics and pyrethroid pollution using the pyrethroid concentrations in stream sediments that were quantified by the California State Water Resources Control Board's Stream Pollution Trends (SPoT) Program. Due to complex interactions among those watershed characteristics and possible non-linear relationship between the characteristics and the pyrethroid concentrations, alternative statistical methods such as partial least square regression and random forest models are considered. In addition, several variable importance ranking methods are explored in order to reach a robust conclusion. The most influential characteristics identified in this study can be used to build mechanistic models and guide the design of future monitoring efforts.

**WP232 Object-to-Mouth Exposure of Residents to Pesticides: What Isn't in the USEPA SOP**

*S. Slade, Blankinship Associates Inc*

The USEPA Standard Operating Procedures for Residential Pesticide Exposure (SOP) (2012) addresses non-occupational exposure to residentially applied pesticides through various routes of exposure. Of the exposure pathways discussed in the SOP, the methods for evaluating incidental ingestion of residues by a child mouthing an object previously sprayed with pesticide are limited. Unlike assessing hand-to-mouth transfer of pesticide residues, the SOP as published does not consider environmental degradation on common lawn objects such as tire swings, toys, and tables. The equation for non-dietary ingestion of pesticide residues on lawn objects would be improved by including a degradation factor in a manner consistent with hand-to-mouth assessment methodology. In this presentation, approaches for modeling dissipation of pesticides on lawn objects will be presented, with an emphasis on the strengths and limitations of each technique.

**WP233 Occurrence and distribution of pesticides in surface waters of California**

*X. Zhang, California Department of Pesticide Regulation / Environmental Monitoring; Y. Luo, N. Singhasemanon, Y. Xie, California Department of Pesticide Regulation*

For decades, state and federal agencies, as well as research institutions in California, have been collecting water and sediment samples for the purpose of pesticide analysis. A wealth of monitoring data has been generated and stored in the California Department of Pesticide Regulation's (CDPR) Surface Water Database (SURF). This study aims to characterize the overall occurrence and distribution of pesticides in the surface waters of California. Various statistical learning methods and GIS tools are used to assess spatial and temporal trends of pesticides in the surface water. Important factors associated with the detections of pesticides will be identified. The results generated by this study will provide an indication of California's aquatic ecosystem health, as defined by relative risks of pesticide active ingredients and degradates to sensitive aquatic species. Moreover, the results will serve to guide future monitoring efforts in California.

**WP234 Buffer Zone Determination Procedures used by the California Department of Pesticide Regulation's Air Program**

*K. Heal, A. Tuli, K. Alstad, M. Kandelous, California Department of Pesticide Regulations / Environmental Monitoring*

A variety of computer simulation models are used by the Air Program at the California Department of Pesticide Regulations (CDPR) to determine buffer zones during agricultural pesticide applications. A buffer zone is described as an area that surrounds a pesticide application block in which certain activities are restricted for a specified period of time to protect human health and safety from existing or potential adverse effects associated with a pesticide application. The models currently used by CDPR's Air Program to determine buffer zones are Agricultural spray Drift model (AgDrift), Industrial Source Complex Short Term Version 3 (ISCST3), and American Meteorological Society/Environmental Protection Agency Regulatory Model (AERMOD). AgDrift is a unique dispersion model that combines Lagrangian trajectory algorithms which characterize the pathway of various sized droplets, with Gaussian deposition algorithms which summarizes all non-evaporated droplets hitting the ground. AgDrift has the capability to estimate downwind deposition of spray drift from agricultural applications and off-site deposition of liquid droplets. ISCST3 and AERMOD are both steady-state Gaussian plume models that can be used to estimate off-site air concentrations associated with pesticide applications. ISCST3 was designed to support the EPA's regulatory modeling programs, and has been used by CDPR as a regulatory tool for air dispersion modeling to develop buffer zones since 1991. In contrast to ISCST3, AERMOD allows for advanced plume dispersion simulation by accounting for boundary layer turbulence and scaling concepts leading to superior performance over previous air dispersion models. As of December 9, 2006, AERMOD replaced ISCST3 as the USEPA recommended dispersion model, and has been recently adopted by CDPR's Air Program. This poster intends to present and explain the various modeling procedures employed by the Air Program for buffer zone determination. Simplified examples will demonstrate each of these modeling approaches.

**WP235 Potential environmental risks associated with pesticide inert ingredients in California**

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California's Pesticide Use Report (PUR) database, queried with the PURwebGIS tool (purwebgis.ucdavis.edu), documents 343 million lbs of pesticide products used in 2015, including 191 million lbs (56%) of active ingredients (AI). The remaining 152 million lbs (44%) are termed "inert ingredients" (inerts), a designation referring only to their lack of specific pesticidal properties and not to any potential risks. The extensive literature on environmental risks of AIs contrasts with very few studies on potential risks of inerts. Pesticide product MSDS give partial listings of inert ingredients. A survey of MSDS for ~1,300 products, comprising >95% of all lbs of product usage in PUR from 2011-2015, revealed hundreds of different pesticide inerts and their formulation proportions. We conducted a full PUR query of inert usage in California for 2011-2015, and analyzed inert usage data in light of potential environmental risks. One interesting group of chemically-related inerts are "Solvent naphtha (petroleum), heavy/light aromatic" (CAS: 64742-94-5 & 64742-95-6) and their C7-C12 aromatic hydrocarbon components, including the carcinogen naphthalene. They are neurotoxic to vertebrates, with aquatic $LC_{50}/EC_{50}$ values of 1-10 ppm for *Daphnia* and various algae, and were present in over 150 different products with 59 different AIs from 2011-2015. In 2015, they accounted for ~3 million lbs of usage and 11.3 million acres treated statewide, including many different crops throughout the state and the growing season, including the rainy season. This study surveys the usage of these "Solvent naphtha" inerts in the Sacramento River watershed (~300,000 lbs used in 2015) since 2011. After input of relevant physico-chemical properties (Koc, half-life, etc.), SWAT analysis was used to simulate their expected environmental fate according to field applications, focusing on potential loading to surface water sources. The results will demonstrate, for the first time, the environmental fate and potential environmental risk assessment of pesticide inert ingredients, and highlight the importance of including potentially high-risk inerts, along with AIs, in environmental monitoring programs.

## Effective Science Communication: Case Studies and Lessons Learned

### WP236 Applying a Translational Science Framework in Environmental Toxicology

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The problem formulation stage of ecological risk assessment has long identified the importance of broad engagement of scientists, stakeholders and decision-makers in assessing and managing risks associated with environmental problems but, in practice, mutual and productive participation is often lacking. In some fields, such as medicine and ecology, recognition of the need for more inclusive engagement has led to the emergence of translational science, an approach in which scientists, stakeholders, and decision-makers work collaboratively to facilitate the development of actionable, decision-relevant science. The underlying framework is based on the incorporation of sound science, socio-economic conditions, and political and public perspectives with the goal of promoting more inclusive decision-making ideally leading to improved policy formation and more effective management practices. While sharing many similarities to the traditional ecological risk assessment framework, it differs in the that it is based on participation of, and input from, all stakeholders at all points and in the application of emerging tools, such as scenario analysis and bow-tie risk assessment, that themselves incorporate collaborative approaches to problem solving and risk identification. In this presentation, I will introduce the concept of translational science, presenting a framework in the context of environmental toxicology; identify similarities and differences from the more familiar ecological risk assessment framework; and provide examples of its application using examples drawn from a scenario analysis exercise conducted to assess contaminants in the Great Lakes and hypothetical applications based on bow-tie risk assessment.

### WP237 Doing Science with a Marketing Plan: Developing the Rapid Benefit Indicators Approach

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Many scientists work diligently to produce tools and resources that can be used to address real world problems. However, a disconnect often occurs between the creation of these scientific outputs and their use by intended stakeholders. Some reasons for this disconnect include limited involvement of users throughout the development of new products and a lack of marketing effort. Several approaches are currently being used by scientists and government agencies to address this problem, including Structured Decision Making and Translational Science. These approaches stress the importance of working with stakeholders throughout the entire process of developing a science product, from problem formulation, through initial planning, all the way to user support of the product. Scientists are often unfamiliar with or untrained in the concept of "marketing" their research products, but it is something that is vital to getting their work into the hands of those who can use them. We used a marketing plan based on the principle of the 4 Ps+1 (product, promotion, price, place + partnerships), during the development of the Rapid Benefit Indicators (RBI) approach, a resource created to help stakeholders assess the societal benefits of ecological restoration using non-monetary indicators. The RBI grew out of stakeholder discussions around the need for a user-friendly set of tools to help make decisions about what sites to target for marsh restoration. We worked with stakeholders through the entire process, from initial design to the development of a web page, a manual, an Excel-based tool, and a GIS-based tool. The marketing plan helped us to target stakeholders for initial discussions, reminded us to keep in touch with stakeholders through the process, and to get the word out to potential users when the product was finished. The approach worked, as evidenced by the fact that in the first year we had over 1,500 page views, over 400 downloads of the Excel checklist tool, and over 300 downloads of the manual. The RBI is also being hosted on several tool compendium web sites, and has been presented at a number of conferences and meetings. In the end it does not matter exactly what approach you use to market your products. What matters is that you have a plan to involve your stakeholders in the development of your products, from start to finish.

## Systems Approaches

### WP238 Sustainability of Struvite Precipitation Systems in Wastewater Treatment

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Current use rates of phosphorus have created concern for the sustainability of global phosphate reserves, and research has begun focusing on ways to recover and reuse phosphorus. Wastewater has gained appeal as a viable resource for phosphorus recovery, and the precipitation of magnesium ammonium phosphate ($NH_4MgPO_4$ $6H_2O$), or struvite, has emerged as viable technology. The intention of this technology is twofold, to prevent unwanted precipitation of struvite in pipes and on equipment in wastewater treatment plants, and to recover a valuable phosphorus product that can offset conventional fertilizer production. This research focuses on evaluating the environmental impact of struvite precipitation systems through the comparison of life cycle assessment studies. Understanding how struvite precipitation systems fit within the context of wastewater treatment, and what added or reduced environmental impacts they bring provides an opportunity to assess the technology as a whole. Literature shows two main schemes of struvite precipitation in wastewater treatment, from a urine source separated stream, or on a side stream within a conventional wastewater plant. Most studies show the greatest contribution to impacts coming from additional energy or chemical requirements. However, specific improvement or decline in environmental impacts depends on the characteristics of the particular system and environment analyzed in addition to boundary parameters such as the inclusion or exclusion of infrastructure construction and an offset of conventional fertilizer.

### WP239 Upcycling Waste to Create a Better Battery Anode using Life Cycle Assessment

*A. Hicks, University of Wisconsin, Madison / Civil and Environmental Engineering*

Increased battery storage is necessary as society shifts from fossil fuels to renewable electricity sources. However, increased battery usage comes with its own set of environmental impacts. Alternative anode materials for batteries, instead of utilizing battery grade graphite, have been a recent area of research. This work presents two synthesis processes for generate battery grade graphitic anode material from waste packing peanuts. Two synthesis processes are investigated, one using an inert gas environment and the other using a vacuum, to generate graphitic anode material, and their relative environmental impacts of quantified utilizing life cycle assessment. Life cycle assessment is a systematic tool for determining the environmental impact of a product or process throughout its lifetime. Often when waste products are utilized, such in the case of plastics, the resulting end product is inferior in some aspects compared to a product generated from virgin feedstocks. In this instance, however, the packing peanut derived graphitic anode material performed better in charge recharge cycles than the conventional battery grade graphite. This is due to the disordered nature of the derived graphitic material, compared to the ordered nature of graphite. This suggests, that in this instance the anode material produced from a waste product is actually superior to the virgin standard. With respect to environmental impact, in the case of the inert gas derived anode material, the greatest contribution was due to the quantity of gas utilized. Whereas for the vacuum derived material, it was due to the electricity consumption, and in turn heavily influenced by the source of the electricity. This work suggests that in some instances of utilizing waste materials that the functionality may be increased compared to using virgin materials. Life cycle assessment was utilized to evaluate the environmental hotspots of the synthesis processes utilized for the graphitic anode materials.

## Policy, Management and Communication

**WP240 A comparative review and analysis of tentative ecological quality objectives (EcoQOs) for protection of marine environments in Korea and China**

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Ecological quality objectives (EcoQOs), as tools for implementing ecosystem approach, have long been acknowledged to protect the marine ecosystems and fisheries in regional seas through joint efforts by surrounding countries over the past decade. The present review analyzed the best available meta-data relating to the five ecosystem elements that were recently proposed by the Northwest Pacific Action Plan to evaluate the current status of coastal ecosystem health in marine environment of the Yellow Sea. We suggested the six tentative EcoQOs among five ecological quality elements including: 1) biological and habitat diversity; 2) invasive species; 3) eutrophication; 4) pollutants; and 5) marine litters. Environmental status were assessed by comparison with the world average values, existing environmental standards, or reported values of other regional seas. Results of analysis revealed that among the six tentative EcoQOs, two target objectives to marine biodiversity and concentrations of nutrients (viz., DIN and DIP) were met towards good environmental status. Whilst, three EcoQOs relating to hypoxia and red-tide, pollutants (persistent toxic substances and metals), and marine litters (including microplastics) did not meet and one relating to invasive species could not be judged due to insufficient data sets. The biggest weak point for developing suitable EcoQOs and assessing status of ecosystem health could be insufficient meta-data sets available and/or differences in chemical analyses and biological identification methods between the countries. Thus, the cooperation of neighboring countries, viz., Korea and China for the Yellow Sea, is necessary for the ecosystem based management of our regional sea in the future. Overall, this first time review for the target tentative EcoQOs in the Yellow Sea regions encompassing coasts of Korea and China would be provide better understanding of the current environmental status and ecosystem health.

**WP241 Forecasting the international process of validating New Approach Methodologies (NAMs) in ecotoxicology: Results from a Policy Delphi study**

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Validating New Approach Methodologies (NAMs) holds the promise of overcoming the limitations with current approaches in regulatory ecotoxicity testing. As time, cost and ethical pressures are mounting towards changing regulatory practices centered on whole animal studies (so-called "kill them, and count them" studies), the global regulatory ecotoxicology community is struggling to find the right way to leverage the desirable properties of NAMs in an appropriate way in their daily work duties. What are the future scenario options for validating the use of NAMs? How desirable and feasible are these options for the future? How much do they – or do not – reflect a consensus in the global regulatory ecotoxicology community? What are the foreseeable impacts and consequences of these options? These are the questions that this poster will address. Building on our previous survey and documentary research, we will elicit the opinion of the world's top NAM experts to provide innovative answers to these challenging problems. We will do so through a Policy Delphi exercise. A Delphi is a group communication process that solicits judgments through sequential questioning interspersed with summaries and feedback derived from previous responses. The Policy Delphi offers a virtual and anonymous, deliberative and analytic participatory group process designed to: 1) integrate the knowledge, values and experience of a group of people representing different areas of expertise; 2) advance social and organizational learning; 3) map consensus and dissent; while 4) minimizing social group dynamics during deliberation. The Delphi is well suited to processes that require input from multiple, different, and often conflicting points-of-view since it allows participants to respond on their own time, to share their opinions without reputational risk, and to contradict others who may occupy a more powerful social position. The steps generally involved in a policy Delphi are: 1) the formulation of the issues; 2) exposing the options; 3) determining initial positions on the issues; 4) exploring and obtaining the reasons for disagreements; 5) evaluating the underlying reasons; and 6) re-evaluating the options. This Delphi exercise will offer opportunities for social learning, which is crucial for effective rational deliberation. It may also uncover disagreements and facilitate the examination of complex problems.

**WP242 QSAR Validation for Pesticide Registration in South Korea**

*J. Ock, Seoul National University of Science and Technology; K. Kim, Seoul National University of Science and Technology / Environmental Engineering*

In South Korea, 3,066 pesticides including organic pesticides were listed under the Enforcement Decree of the Agrochemicals Control Act. Non-animal testing is recommended for toxicity test in chemical regulation, The Quantitative Structure-Activity Relationship (QSAR) models have been increasingly utilized to predict toxicity end-points by using physico-chemical properties (e.g. molecular structure) of chemicals of target.
To examine the applicability of QSAR into the prediction of fish acute toxicity of pesticides, the Ecological Structure Activity Relationships (ECOSAR, v2.0) providing various information of organic compounds, was used for predict 96h LC50, which compared with experimental LC50 values identified from National Chemicals Information System (NCIS). Our results suggest that QSAR could be the alternative tool of toxicity testing with animal model QSAR in pesticide registration. Further toxic end-points should be assessed to increase the applicability and usage on registration procedure of chemicals.

**WP243 Sustainability Index: A KPI for Industry**

H. Plugge, *Verisk 3E*

Sustainability is often described as an essential pillar of environmental stewardship. How can we track how sustainable an enterprise is? For chemical manufacturers and especially distributors and formulators, it can be a minefield of different definitions and methodologies. Here we have defined sustainability as optimizing risk, by first tackling the big risks followed by lesser risks. How does one go about defining let alone calculating “risk” for a chemical distributor with often thousands of products? Given that hazard and risk are often used interchangeable (they are not) one first needs to discuss hazard assessment: the art of defining a chemical as a hazard regardless of concentration (Hence 60 ppm formaldehyde in a pear is ignored while 30 ppm in shampoo is hazardous and banned- de facto regulation). Risk in its simplest form equals the product of hazard and exposure. The environment long has had Key Performance Indicators aka environmental standards or thresholds. Thus the presence of 2 mg/L of a metal is a KPI. Such KPI’s are always single media and single chemical based. How is a formulator with 15000 plus products (aka mixtures) to produce a KPI or Sustainability Index? Defining sustainability as optimizing risk allows one to provide an enterprise wide risk screening estimate. Given that this involves summing over thousands of mixtures, how does one go about calculating a single KPI for either health or environmental risk? First of all this will be a quantitative risk screening estimate with lots of simplifying assumptions. Exposure can be thought of as total inventory present i.e. the quantity of chemicals within an enterprise. Hazard is more tricky. Using our 3E GreenScore methodology one can calculate a hazard metric based on 26 parameters which is quantitative and dimensionless, thereby allowing summing of effects across chemicals. Risk = hazard X exposure summed across all chemicals in the inventory. A KPI can now be calculated as excess risk per facility or probably better as excess risk per unit of inventory e.g. risk/kkg. The optimum KPI ideally would be 1, so its proximity to 1 can be measured over time as sustainability and hence the KPI improves. Such KPI’s can be calculated for health and environmental hazard (it is not recommended they be combined as they tend to trend very differently.) It should be noted here that these KPIs can be used for optimizing risk and delineating sustainability using hazard based approaches.

**WP244 The predictive lead emissions of lead-acid batteries toward 2030 in China based on the lead life cycle and its metabolism**

S. Chen, *S. Li, Z. Lian, Beijing University of Technology / College of Environmental and Energy Engineering*

Lead-acid batteries (LABs), widely used batteries in cars and electric vehicles, have occupied a very important position in batteries industry for more than a decade. In this study, we analysed the lead metabolism in LABs industry systematically. We obtainedthe LABs production, lead demand of LABs, secondary lead production potential of LABs and lead emission of LABs before 2015 through literature collection in China. It wasfound that the LAB production increased dramatically before 2015. Then, we proposed three scenarios for LABs production: lead demand of LABs, secondary lead production potential of LABs and lead emission of LABs toward 2030. The results showed that the LABs production would continue to grow under the high scenario, reach a peak under thebaseline scenario and then decrease under the low scenario. As for thelead demand of LABs, it will goto a peak around 2020 under the high scenario and baseline scenario, then bereducedto 6.16 and 3.84 million tonnes toward 2030, andit will continue to decrease to 2.63 million tonnes toward 2030 under low scenario. The peak value of secondary lead production potential of spent LABs willbe 2 or 3 years later than the lead demand of LABs and it will continually reach the lead demand of LABs in the year. The total lead emission will reach peak in 2020 and then decrease and the total lead emission under the three scenarios will be 2.16, 1.67 and 1.36 Mt in 2020 and reducedto 1.67, 1.26 and 0.78 Mt toward 2030, respectively. *Keywords:*Leadacid batteries production, Lead demand, Secondary lead, Lead emission

**WP245 Verification of QSAR models for persistence and bioaccumulation of SVHCs**

J. Moon, *Seoul University of Science & Technology; J. Ra, Korea Institute of Industrial Technology; K. Kim, Seoul National University of Science and Technology / Environmental Engineering*

SVHCs (Substances of Very High Concern) regulated by the European Chemicals Agency include the substances classified as Carcinogenicity, Mutagenicity, toxicity for Reproduction (CMR) and persistence or bioaccumulation. The persistence and bioaccumulation of chemicals determined by experimental or non-tested methods are very important indicators related to chemical regulations. Among non-test methods, the Quantitative-Structure Activity Relationship (QSAR) model is a method of predicting physico-chemical properties or toxicity based on the structure of the chemical, and is also used to evaluate persistence and bioaccumulation. However, since QSAR is currently not fully validated, incorrect predictions of QSAR might lead to applicability limit. This study will validate and analyze the KWWIN, BIOWIN, BCFBAF, and HYDROWIN, which are used to predict the persistence and bioaccumulation of chemicals. The verification and analysis of the QSAR models will be performed by comparing the model predictions with the actual values. Target substances will be categorized as “SVHCs classified persistence or bioaccumulation” and “Substance not classified persistence or bioaccumulation” and will identify differences in accuracy and limitation between two groups. Additionally, differences will be identified depending on the structural properties of the chemical. Further studies are needed for various non-tested methods such as read-across, categorization as well as various QSAR models.

## Engineering, Remediation and Restoration

**WP246 A meta-proteomic study of polystyrene degradation by gut microbiota of *Tenebrio molitor* larvae**

A. Navlekar, *Texas Tech University; D.L. Carr, Texas Tech University / Biological Sciences*

In the last 50 years, there has been a rapid increase in plastic usage in almost every sphere of life. Polystyrene (PS) or Styrofoam, as it’s more commonly known, has been used in various products like cups and packing material and also as a raw material and solvent in the processing of polymers. Its uncontained production, constant daily usage and incorrect disposal have led to grave environmental problems including destruction of wildlife habitats and bioaccumulation in food chains. Until very recently, it was assumed that polystyrene was non-biodegradable. A study conducted in 2015 showed the ability of *Tenebrio molitor* larvae to degrade polystyrene; the ability was attributed to their gut microbiome (Yang et al., 2015). The researchers concluded that the degradation must occur due to secretion of enzymes by their gut micro-organisms. We recently completed a metagenomic study of polystyrene (PS) degradation by *Tenebrio molitor* (larvae of the Mealworm beetle) where we showed that the native gut microbial community of *Tenebrio* itself can degrade polystyrene without much change to the community composition or structure. To further elucidate the proteins involved in this degradation, we conducted a metaproteomic study of gut microbiota of *Tenebrio*. In this study we report on the metaproteomic diversity and identification of major protein groups that the gut microbiota of mealworms produced when fed with Styrofoam as their only food source and compared them to mealworms fed a normal bran diet. Proteins were extracted from the guts of *Tenebrio* larvae (standard diet vs. PS-fed only) using cytosolic and membrane protein extraction buffers. The extracted proteins were separated on the basis of pI and hydrophobicity using a Two-Dimensional Protein Fractionation instrument. Selected aliquots were then trypsonized and passed through a mass spectrometer with Orbitrap mass analyzer for identification by their mass-to-charge ratios. Results from this study contribute to understanding of the functionality of this mixed gut community that enables *Tenebrio* to degrade PS successfully. The broader impact of this study lies in determining microbial proteins with homological similarity to existing proteins, especially those that can be mass produced. The use of mass produced homological proteins/enzymes for plastic recycling, one of the most intractable problems of human society today, are expected deliverables from this study.

**WP247 Adsorption kinetics for the removal of dyes by millet and rice husk bio-waste materials in aqueous medium**

*A. Udeozor, Evangel University Akaeze Ebonyi State / Bio-chemistry; O. Mbonu, Federal Polytecnic Afikpo Uwana / Biochemestry*

Colouring materials that gives desirable colours to our everyday activities and commodities are called Dyes. Dyes can be Cationic (basic in nature), Anionic (acidic in nature) or Amphoteric (exhibiting both acidic and basic character). There is hardly any industry where dyes aren't required either for beautification or for protection ranging from Children's toys, Household appliances to fabrics. Due to the ubiquitous nature of dyes, they constitute environmental pollutants that pollute water bodies. This research investigates the removal of the dye, methyl red (MR) and methyl blue (MB) from aqueous environment using two biosorbent materials, namely, Millet and Rice Husk. These two selected biosorbents were studied under varying experimental conditions such as: effect of Contact time, Temperature, pH, Dye concentrations among others. The results of these experiments were modeled using standard Physical Chemistry Sorption Kinetic Isotherm Models. The result of these models shows that the two biodegradable locally available materials fitted some of these models like Pseudo first and Pseudo second orders, Langmuir and Freundlich isotherm. The calculated Sorption maximum capacities are 56.67% (2.67mg/g), for MilletHusk and 52.04% (2.57mg/g) for Rice Husk at 318K. It was observed that Sorption increases with increase in standard temperature in Kelvin for the two biosorbents studied. Various Isotherm models evaluated showed that the two Biosorbents have significant potentials as biodegradable sorbents for the removal of Methyl orange and Methyl blue colours from aqueous environments.

**WP248 Biodegradation and Final Solution Toxicity of Mature Fine Tailings from Oil Sands Processing in Fort McMurray, Canada**

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Alberta Canada contains the third largest oil sand reserve in the world, producing about 2.3 million barrels of oil per day. Oil sands are a mixture of sand, clay, small amounts of water, and the viscous hydrocarbon bitumen. Mature Fine Tailings (MFTs) are the byproduct of oil sand refinement, as bitumen is separated from the sand and clay. MFTs contain many different chemicals and toxic material, such as naphthenic acids, which are potential endocrine disruptors, and polycyclic aromatic hydrocarbons, some of which are carcinogens. These contaminants are of major concern to the environment because of their toxicity and persistence. It has been found that the patented microbial consortium BioTiger™ (BT) has the ability to cometabolically biodegrade specific chemical components that are found in MFTs, including hexanoic acid and phenanthrene. The purpose of this project was to determine BT could successfully remediate the toxicity of MFTs collected from Fort McMurray, Canada. Test solutions were prepared by combining BT with MFTs in an aqueous solution. The BT/MFT sample was then split; half was immediately cooled to 4°C to slow/halt bacterial activity; and the other half was allowed to incubate for 7 days at room temperature. Acute toxicity tests were conducted on each solution with the freshwater organism, *Daphnia magna*. Results indicated that incubation with the BT significantly increased the toxicity of the MFT solution. Chelation with EDTA did not significantly reduce toxicity, indicating that dissolved metals were not the ultimate source of toxicity despite their high concentrations in the MFTs.

**WP249 Characterization of Sorption Mechanisms for Enhanced Microbial Remediation Processes of Uranium**

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Based on our batch experiments, microbial remediation studies that include *Bacillus spp*, and *Burkholderia* sp. may immobilize uranium transport by forming uranium-bacteria complex in the presence of phytate. Our current column studies were conducted to demonstrate that without the uranium-bacteria complex formation, uranium was able to be transported in the subsurface soil with through mediation of *Burkholderia* sp. Specifically, uranium transport was observed in three different scenarios: (1) uranium pre-deposited in the soil and then flushed with *Burkholderia* sp., (2) uranium and *Burkholderia* sp. mixture being introduced to the column, and (3) uranium and *Burkholderia* sp. being introduced at the same time but separately. Column transport experiments were conducted by acrylic columns with 3.0-cm ID ´ 10.0-cm length. The soil was packed in the column through $CO_2$ solvation to eliminate air pockets. For all above three scenarios of uranium introduction, 10 ml uranium at a concentration of 10 ppm was used. For *Burkholderia* sp. application, cell aliquote at a concentration of $10^8$ cells/ml was used. Studies with *Bacillus spp* and other competent bacterial strains will be demonstrated as well. We envision that the outcome of this study could provide a framework for: 1) long-term monitoring of U at DOE waste sites; 2) provide a tool for evaluation of natural attenuation-based processes; and 3) present research opportunities for enhanced attenuation approaches for radionuclide and heavy metals at DOE sites, and other uranium-contaminated sites

**WP250 Characterizing the Groundwater-Surface Water Interface Pathway at Hazardous Waste Sites using Two Pore Water Sampling Methods**

*R. Zajac, CH2M; J. Johnson, M. Fulkerson, B. Fishwild, A. Baseman, Jacobs*

The groundwater-surface water interface (GSI) is an important pathway to evaluate, as contaminated groundwater may discharge into surface water, and can impact aquatic ecological receptors. This case study compares the use of two different pore sampling methods to characterize pore water at hazardous waste sites. The well point method and push point method were used at two different sites, located in the Midwest and Southeast, to assess the GSI pathway. Each site presented a different objective and need for GSI data. At the Midwest site where the well point method was utilized, the objective was to delineate a VOC groundwater plume and conduct a screening-level ecological risk assessment for aquatic receptors. At the Southeast site, where the push point method was utilized, the objective was to assess the potential for the discharge of VOC-contaminated groundwater into the creek, which also supported an evaluation of an existing pump and treat groundwater treatment system upgradient of the creek. This case study presents the data needs to evaluate potentially complete GSI pathways, information on each sampling method, and demonstration on how the data collected at each site was used to aid in the investigation and decision-making process.

**WP251 Chemistry's role in understanding and remediating a Superfund Site that's home to numerous abandoned mine and mill sites**

*E. Naylor, A. Hughes, Maul Foster & Alongi*

The East Fork Ninemile (EFNM) Basin, an approximately 3-mile-long watershed just outside Wallace, Idaho, and within the Bunker Hill Superfund Site, is burdened by numerous abandoned mine and mill sites. The basin contains over a million cubic yards of mine waste laden with lead, zinc, and cadmium, found primarily in consolidated mine waste piles adjacent to EFNM Creek. Physical and chemical processes affecting the mine waste piles have resulted in contamination of the watershed's native soils, groundwater, surface water, and sediments, posing a risk to human as well as terrestrial and aquatic ecological receptors. A Record of Decision issued by the USEPA requires remedial action at several sites in the EFNM Basin. Iterative investigative work has been conducted throughout the EFNM Basin to create conceptual site models that are used to direct and prioritize site remedial actions. Predesign investigations are conducted at mine waste piles to determine the nature and extent of contaminants of concern in all media. Subsequent remedial design investigations fill environmental data gaps and provide designers with geochemical, geotechnical, and agronomic parameters at each pile. Recently, an area-wide investigation was designed and implemented to establish how/where groundwater and surface water may be loading metals to the EFNM Creek from known sources, identify possible unknown sources, and provide baseline to evaluate the effectiveness of future remedial activities. The findings of ongoing predesign and remedial design investigations have resulted in the removal of 650,000 cubic yards of mine waste and impacted native soils and remedy effectiveness monitoring has begun in some of the removal areas. The area-wide surface and groundwater investigation conducted has identified a significant nonpoint source of metals loading into the EFNM Creek that was previously unknown/unanticipated and conversely, shown that a large mine waste rock pile is not a significant source of loading. This discovery has helped complete the basin-wide conceptual site model. These findings will require additional investigative work, the results of which will guide future remedial actions and remedy effectiveness monitoring in the EFNM Basin.

**WP252 Development of passive sampling approach for assessing atmospheric Polychlorinated biphenyls (PCBs)**

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Volatile losses of hydrophobic organic contaminants (HOCs) such as polychlorinated biphenyls (PCBs) during placement and disposal of contaminated sediments are a continual cause of concern to confined disposal facilities (CDFs) and its environs. Presently, conventional method such as the high-volume sampler is employed to constantly monitor the level of atmospheric HOCs surrounding the CDFs. This method of sampling is labor intensive, costly and incapable of providing long term averaged concentration of contaminants. To address these limitations, passive sampling approach is being explored as a potential air sampler. Preliminary laboratory time series experiments were conducted to evaluate polydimethylsiloxane (PDMS ~34 µm) and Polyoxymethylene (POM-76 µm) as passive air samplers for HOCs, and to assess the potential of the chosen air sampler to contaminated sediment collected from Indiana Harbor and Canal, Confined Disposal Facility (IHC- CDF). The laboratory evaluation was conducted by exposing polymeric passive samplers in the airspace over an aqueous solution to which was introduced a mix of PCBs or to a slurry of sediment collected from the IHC-CDF. Uptake on the samplers was determined by high-performance liquid chromatography (HPLC) and gas chromatography, triple quadrupole Mass spectrometer (GC-TQ). The results suggest that POM gives a longer exposure time averaged concentration compared to PDMS, and that POM is generally not responsive to short term losses prevalent in the system. Analysis of experiments with slurries using sediment from the IHC-CDF showed a consistent uptake of PCBs on POM and their POM-air partition coefficients measured ranged from 6.35 to 7.91 log units. The partition coefficients measured differs from literature values by 0.1-0.4 log units and suggests an underestimation of the partition coefficients at 28 days especially for higher molecular weights PCBs. The method was then tested with preliminary application of POM deployed at 10 stations around the IHC-CDF over 42 days to measure air concentration of PCBs. The results show generally low air concentrations around the site with total PCBs below 10 ng/m$^3$. The passive sampler measured air concentrations of specific congeners that were very similar in magnitude to those measured by high volume air sampling at the same locations. In summary, passive sampling exhibits the potential to be an excellent alternative means of measuring PCB air concentrations around the CDFs.

**WP253 Effect of Laser Stimulation on Plant Growth and Uptake of Cadmium in Indian Mustard (*Brassica napus*)**

*B. Rimal, Texas Tech University / Biological Sciences*

*Brassica napus (*Indian Mustard) is often found as a primary colonizer of disturbed lands including mine tailings, and is reported to accumulate high concentrations of Cadmium while also producing high concentrations of metal chelators like succinic acids at its root zone. We conducted an in-vitro experiment to observe the germination, biomass accumulation and cadmium uptake potential of laser stimulated and non-stimulated Indian mustard grown in half MS-medium for two weeks. Cadmium spiked medium were maintained at 20 ppm and 40 ppm cadmium chloride concentrations. 3 replicate bottles with 6 seeds for each treatment were maintained at 20° C, 16:8 light/dark cycle and 275 mmoles/m$^2$/s irradiance. Destructive sampling of the plants was done after 2 weeks for quantification of accumulated biomass and cadmium uptake at plant roots and shoots. No significant difference was observed in germination rate between laser stimulated and non-stimulated seeds grown in both cadmium spiked and non-spiked medium. Laser stimulated seeds grown in medium spiked with 20 ppm cadmium produced significantly higher root, shoot and total biomass . Laser stimulated seeds grown in medium spiked with 40 ppm cadmium produced significantly higher root biomass and laser stimulated seeds grown in medium with no cadmium contamination produced significantly higher total plant biomass and shoot biomass.

**WP254 Effectiveness and Optimization of Remedial Actions for Risk Management of Perchlorate-Impacted Groundwater**

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Historical operations at a former Navy facility near McGregor, Texas lead to perchlorate contamination in surface water (up to 5,600 ppb), groundwater (91,000 ppb), and springs (22,000 ppb) in a 750-acre watershed. To control contaminant migration off-site and limit surface water contamination from groundwater, Interim Stabilization Measures (ISM) and Institutional Controls (IC) were put in place. ICs included land use controls and a deed notice to limit property use to commercial/industrial (i.e., non-residential), establishment of a Plume Management Zone, and a deed notice to establish a landfill cap (Class II/III landfill). ISM include on-site treatment of impacted soils comprised of a lined soil treatment system with anaerobic landfarming with citric acid, nitrogen and phosphorus, and soda ash amendments. In addition, a pump and treat system was installed to eliminate the groundwater to surface water migration pathway and prevent continued offsite plume migration. In addition to the ISM, additional remedial actions were conducted at the southern end of the Plume Management Zone that included passive in situ biotrenches/biowalls and bioborings amended with mushroom compost, woodchips, and gravel mixed with emulsified vegetable oil. These treatments effectively reduced perchlorate concentrations in groundwater at the Site; as of 2018 the mass of perchlorate in groundwater has been reduced by 80% from 2000 mass calculations. This mass reduction, along with updated ecological hydrogeologic, and hydraulic studies is allowing the Navy to consider alternative remedial measures and optimization. The current optimization is focusing on a completely low cost passive in situ system designed to remediate a smaller, higher concentration on-site plume segment since off-site concentrations in both groundwater and surface water are below regulatory limits.

**WP255 Efficiency of Columbia Wetlands in Removing Pharmaceuticals and Personal Care Products from Treated Municipal Wastewater**

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Pharmaceuticals and personal care products (PPCPs) represent pollutants of emerging concern, originate from direct human disposal and other personal care use before disposal in the wastewater systems. Several classes of PPCPs that have endocrine disrupting activity such as fluoxetine (antidepressant), erythromycin (antibiotics), iopromide (X-ray contrast), estrone (estrogen), triclosan (antibacterial), and progesterone (endogenous steroid hormone) are persistent and frequently found in effluents from municipal and hospital wastewater treatment facilities. Endocrine disruptive PPCPs can adversely affect normal reproductive, behavioral, immune system, and neurological functions of aquatic organisms. Several studies indicated that conventional wastewater treatment plants (WWTPs) are not able to completely remove these kinds of micropollutants from wastewaters and they are consequently discharged into the aquatic environment. Constructed wetlands have been successfully used as a tertiary treatment process for removal of biological oxygen demand (BOD), total suspended solids (TSS), organic matter, nitrogen, and phosphate from domestic wastewater. However, there are studies showed the effectiveness and efficiency of this low-cost, environmentally friendly tertiary treatment process in the removal of the PPCPs. In this study, we conducted sampling sessions over a period of one year on Columbia wetlands (CWTs) and more than fifty PPCPs compounds in the inflow and outflow have been detected. High removal efficiencies were observed for estrone, azithromycin, tolfenamic acid, and diphenhydramine. From the present study, it can be concluded that Columbia wetlands may provide a complementary treatment option.

**WP256 Evaluation of different biological activities in monitoring of the effects of increasing concentration of petroleum in agricultural soil**

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The resultant effects of the persistent contamination of the soil environment by organic pollutants are known to be deleterious to soil components and by extension, humans. Therefore, bioremediation monitoring is pertinent in ensuring the effective and efficient restoration of soil activities. In this study, soils polluted with varying concentrations of diesel (IC 1, IC 2, IC 3) at initial TPH concentrations of 14785.84 mg/kg, 23859.52 mg/kg and 42134.96 mg/kg, respectively, were bioremediated using rice husk as biostimulant. Different soil biological parameters namely soil enzyme activities (catalase and b-glucosidase), soil microbial biomass carbon (MBC), nitrogen (MBN) and phosphorus (MBP), soil microbial respiration as well as the soil phytotoxicity were used to monitor the bioremediation process. At the end of 56-day study, the degradation rate for IC 1, IC 2 and IC 3 were recorded at 99.1%, 98% and 97.6% respectively. The 1$^{st}$, 2$^{nd}$ and nth-order kinetic equations were used in determining the efficiency of the treatment for the increasing concentrations of diesel polluted soils. The results of 1$^{st}$ order kinetics for IC 1 ($k$= 0.6745 d$^{-1}$, $R^2$= 0.9388); IC 2 ($k$= 0.5738 d$^{-1}$, $R^2$= 0.9287); IC 3 ($k$= 0.6058 d$^{-1}$, $R^2$= 0.9626); 2$^{nd}$ order kinetics for IC 1 ($k$= 8.748×10$^{-5}$ d$^{-1}$, $R^2$= 0.8594); IC 2 ($k$= 4.301×10$^{-5}$ d$^{-1}$, $R^2$= 0.8457); IC 3 ($k$= 3.046×10$^{-5}$ d$^{-1}$, $R^2$= 0.9839); nth order kinetics for IC 1 ($R^2$= 0.9492, $k$= 4.472 d$^{-1}$, $n$= 0.7919); IC 2 ($R^2$= 0.9394, $k$= 6.239 d$^{-1}$, $n$= 0.7517); IC 3 ($R^2$= 0.9882, $k$= 0.0028 d$^{-1}$, $n$= 0.3082). From the results obtained, all biological activities for IC 1 except MBN were most responsive to the rice husk treatment than those of the IC 2 and IC 3. Improved plant growth was also observed in IC 1 and IC 2, as compared to IC 3, towards the end of the bioremediation study. These outcomes showed that the use of biological parameters is indispensible in monitoring the efficacy of a bioremediation process on contaminated soil.

**WP257 Groundwater-Surface Water Interface (GSI) Investigations used to Guide Risk Management Decisions**

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Historical investigations at an industrial facility in the Eastern United States identified elevated volatile organic compounds (VOCs) in site groundwater. Site-related groundwater data were evaluated to identify potential groundwater plume venting areas in the river adjacent to the facility where ecological receptors might be exposed at the groundwater-surface water interface (GSI). Multiple pore water investigations were conducted to identify potential VOC venting zones and determine if VOC concentrations in sediment pore water potentially pose unacceptable ecological risks to aquatic biota. Each investigation phase involved collection of pore water for VOC analysis using the Trident Probe, a direct-push, integrated conductivity/temperature sensor and GSI sampler. The first phase of the investigation included collection of pore water opposite areas of elevated onshore groundwater VOCs in 9 site remediation areas. The initial phase confirmed the presence of VOCs in pore water exceeding ecological screening values opposite 6 remediation areas. Subsequent investigations were designed to facilitate additional pore water characterization and delineation in identified venting zones. The Trident Probe data provided key information needed to refine the hydrogeological conceptual site model regarding the relationship of site groundwater with the GSI. Subsequently, the pore water data were used to evaluate potential ecological risks, support ongoing onshore site characterization activities, and to support ongoing risk management decisions. This presentation presents the Trident Probe methods employed, investigation findings, and how the data collected supported risk management decisions.

**WP258 Immobilized fruit and vegetable peels as effective biosorbents for removal of arsenic and heavy metals simultaneously from cocktail solution**

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Contamination of arsenic and heavy metals in drinking water is a globally growing concern as these produce toxicity even at very low levels. Various methods for removal of such non-biodegradable toxins have been proposed, but are likely to be impractical in developing countries owing to their high costs. Biosorption is a cost-effective and convenient method that may be used for decontamination of water by incorporating waste products such as fruit and vegetable peel (FVP) produced in large quantities by food and beverage industries. The surfaces of these FVPs have certain functional groups that provide natural binding sites for the uptake of various ions from aqueous solution. The current study aims to study the potential of a few FVPs such as apple, banana, cucumber, kiwifruit, orange and potato peels as potential biosorbents for removing arsenic, cadmium, chromium, copper, mercury, lead and nickel simultaneously from drinking water. The analysis showed that immobilization makes these biosorbent 'beads' easier to remove from the solution after biosorption as compared to the process of separating FVPs particles dispersed in the solution. In addition, immobilizing these FVPs on sodium alginate 'vehicle', which has biosorbent properties of its own, produced some biosorbents that had significantly higher biosorption% and smaller reaction times as compared to equilibrium times of empty 'vehicles' (2-3 days). The elements in the study demonstrated different affinities for the six FVPs at different time points. Equilibrium biosorption was found to be 21% (As V), 92% (Cd II), 34% (Cr VI), 87% (Cu II), 90% (Hg II), 85% (Pb II) and 80% (Ni II) with minimum biosorbent dose for 0.1 mg L$^{-1}$ cocktail solution. Biosorption could be increased by increasing the FVP dose until equilibrium was attained between ions in solution and that on the biosorbent surface beyond which it was not possible to increase uptake by the unmodified FVPs. This was established by using the same metals in cocktail solution at 1 mg $L^{-1}$.

**WP259 Importance of understanding the toxicity values available for chromium, potentially complete routes of exposure and implications for remediation**

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Often in site characterization investigations, total chromium analysis is performed on soil and water samples, especially if hexavalent chromium (chromium[VI]) was not a chemical used in historical operations. Toxicological values are not available for total chromium, but rather the two valence states: chromium(VI) and trivalent chromium. Commonly, assessors and regulatory agencies default to use of the more conservative chromium(VI) screening to evaluate total chromium soil data and select constituent of potential concern (COPCs). The US Environmental Protection Agency (USEPA) Regional Screening Level (RSLs) for soil and tapwater are primarily based on the oral carcinogenic toxicity values for chromium(VI). However, upon closer examination, the USEPA Integrated Risk Information System did not identify data to support carcinogenicity by the oral route of exposure. Therefore, it may be more be more appropriate to use the non-carcinogenic toxicity value to generate the screening levels used for selection of COPCs and calculation of oral hazards in a human health risk assessment. The chromium(VI) residential soil carcinogenic screening level (target risk of $10^{-6}$) is 0.3 milligrams per kilogram (mg/kg), while the non-carcinogenic screening level (target hazard index of 0.1) is 23 mg/kg (USEPA RSLs tables, May 2018). The increase in screening level may reduce the footprint of potential remedial effort significantly because the screening level based on the non-carcinogenic toxicity value is two orders of magnitude greater than the carcinogenic screening level. While the IRIS did not find evidence of oral carcinogenicity for chromium(VI) it did find evidence of carcinogenicity through the inhalation route. Therefore, it is important to take into consideration the potentially complete exposure pathways identified for a site when conducting a human health risk assessment evaluating chromium as a COPC.

**WP260 Innovations in groundwater remediation driven by extremely challenging, emerging contaminants: 1,2,3-Trichloropropane (TCP)**

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Groundwater can be contaminated with 1,2,3-Trichloropropane (TCP) from industrial and agricultural activities, and very low concentrations of TCP are of concern (CA recently adopted an MCL of 5 ppt), so treatment of TCP contaminated groundwater is an emerging challenge. Since TCP is not removed efficiently or rapidly by established methods of groundwater remediation (e.g., by adsorption to GAC or dechlorination with zerovalent iron), it has become a test case for innovative remediation technologies. We have explored several of these, most notably zerovalent zinc (ZVZ), but also sulfidated nano ZVI, bisulfite-activated permanganate, etc. The dechlorination of TCP by ZVZ is of particular interest because it exhibits very high selectivity for reductive beta-elimination over hydrogenolysis. Molecular modeling indicates that reductive elimination is thermodynamically and kinetically favored, but the zinc also appears to contribute to this selectivity. ZVZ has proven effective for remediation of TCP in several pilot-scale field tests. TCP can be degraded under conditions of several advanced oxidation technologies, but their performance has not been sufficiently promising to lead to field applications.

**WP262 Remediation of Microcystins in Drinking Water Treatment Plant Waters and Sludges with Electron Beam Irradiation**

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Harmful algal blooms (HABs) pose serious threats to human and animal health, and aquatic ecosystems. HABs can occur in any nutrient rich aquatic environment, especially drinking water sources. The cyanobacterium *Microcystis aeruginosa* is commonly associated with HABs and is responsible for producing various hepatotoxic heptapeptides termed microcystins. Of the over 80 variants of microcystins, microcystin-LR (MC-LR) is the most prevalent and the most toxic. Microcystin's biological activity comes from the unusual amino acid (2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (ADDA) which results in the inhibition of protein phosphatase 1 and 2A in humans. Disruption of this pathway ultimately results in liver inflammation, hemorrhaging, acute pneumonia, and has a potential to promote tumor growth. Ozonation is the only known microcystin removal technology for the drinking water industry. Though relatively effective for drinking water, it is not a feasible technology for remediating treatment plant sludges that could have high microcystin concentrations. Due to its toxicity, states like Ohio now classify such sludges as hazardous waste that cannot be landfilled, creating a need for new decontamination or remediation technologies. Our underlying hypothesis is that high energy electron beam (eBeam) irradiation technology, an advanced oxidation/reduction process, could be effective for the detoxification of microcystin-contaminated water, sludges, and sediments. Data suggests that even at low eBeam doses (< 5 kGy), MC-LR is completely destroyed in aqueous samples. Samples dosed with only ~400 Gy were found with less than 1% of microcystin remaining in comparison to untreated samples as determined through LC-MS/MS. Similar results were obtained using the ADDA-specific Enzyme-Linked Immunosorbent Assay (ELISA) as described by EPA method 546. Dosed samples showed a decline in MC-LR to below the 0.15 ppb detection limit of the assay. EBeam technology, which relies on accelerating electrons, does not involve the use of chemicals. Thus, eBeam technology has the potential to be a sustainable and more cost-effective alternative treatment strategy for such applications.

**WP263 The Mechanistic Study of Perfluorooctanesulphonate (PFOS) Adsorption on Nano Alumina**

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The interaction of pollutants with nanomaterials has attracted many attentions due to the extensive application of diverse nanomaterials. In this study, PFOS was selected to investigate its adsorption behavior on nano alumina with different shapes. Firstly, the adsorption kinetic and isotherm of PFOS on alumina nanoparticles (NPs) and nanowires (NWs) were studied, and the thermodynamic parameters were calculated for better understand the adsorption behavior. Then the effects of solution chemistry (including: pH, ionic strength, and humic acid) on adsorption were further studied. Based on these results, the adsorption mechanisms were proposed from perspectives of particle properties, solution chemistry, and aggregation effect. The different aggregation behavior of alumina NPs and NWs were found to be the critical factor for PFOS adsorption, which was further proved by the dynamic light scattering (DLS) experiments. This study is the first to study the aggregation effects on PFOS adsorption on nanomaterials and the results should be useful to identify the important roles of shape and aggregation of nanomaterials for the fate of organic pollutants in environment.

**WP264 Effect of the support on the catalytic activity of Mo catalysts in the hydrodechlorination of PCDD/Fs and dl-PCBs**

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Catalytic hydrodechlorination is an effective method for the removal of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls, similar to dioxins (dl-PCBs) due to the moderate operating conditions required and the relatively low operating costs. However, the catalyst to be used must be resistant to poisoning by hydrochloric acid formed during the reaction. The PCDD/Fs and dl-PCBs are unintentionally generated in combustion processes, where they are present in its gaseous emissions and solid wastes, such as fly ashes. In catalytic hydrodechlorination of organochlorine compounds, although Pd is considered as the most active and selective metal, it has been found that this catalyst is fastly deactivated. The aim of this work is to evaluate the catalytic hydrodechlorination of extracts of PCDD/Fs and dl-PCBs using Mo supported, by wet incipient impregnation, on g-alumina (g-$Al_2O_3$), silica ($SiO_2$) and activated carbon (AC); catalysts were characterized using BET, XRD, $NH_3$-TPD and UV–vis spectroscopy. The initial total concentration of the furans almost doubled the initial total concentration of the dioxins and exceeded by more than 40 times the initial total concentration of the dl-PCBs, The congener that had the highest initial concentration (7971 pg/mL), among the group of furans, was 2,3,4,7,8-PeCDF; therefore, it has a large contribution to the total toxicity of this group (51%). The congener that had the highest initial concentration (3685 pg/mL) among the group of dioxins was 1,2,3,4,6,7,8-HpCDD (1.9%); however, it does not have a high toxic equivalence factor. The congener 1,2,3,7,8-PeCDD has the main contribution (61%), with a concentration of 1203 pg/mL. The congener of the group of dl-PCBs that contributed the most was PCB 126 (1002.33 pg/mL), which has the highest toxic equivalence factor, with a large toxicity contribution (89%). In general, the contribution to the toxicity of furans was 69.1%, dioxins 29.2% and dl-PCBS 1.7%. Reduction in toxicity was slightly higher in dioxins than in furans over the tested catalysts. The catalyst that most reduced the congener with the highest toxicity was MoA (302.77 pg/mL). The order of activity of the catalysts for all the PCDD/Fs congeners was MoA > MoCA > $MoSiO_2$ and for the dl-PCBs congeners was MoA > $MoSiO_2$ > MoCA. The catalytic activity of Mo supported on g-$Al_2O_3$, for hydrodechlorination, is comparable to the literature (71 – 99%).

## Protecting Freshwaters from Salinization

### RP001 Effects of temporal variability of salt disturbance on leaf decomposition and leaf shredder activity

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The heightened presence of road deicing salt in streams, as a result of increased use during winter months, is a disturbance that interferes with aquatic ecosystem functioning. Leaf shredding insects, which are particularly sensitive to salinity in freshwater ecosystems, aid in the decomposition of leaf litter and are an important indicator of the health of streams. Loss of these invertebrates impacts not only the health of streams, but the recreational value of these ecosystems through accumulation of detritus. With the need to balance ecosystem health with retaining human safety during winter months, research evaluating the extent to which deicing salts impact leaf shredding insects and nutrient cycling in streams is needed. The goal of this research was to evaluate how temporal variability of salt delivery within streams affects leaf shredding organisms and leaf litter decomposition. To explore this, Tipulidae larvae were exposed to one of five road deicing salt treatments at 6°C for 33 d in laboratory mesocosms. Treatments included no salt (Control), constant salt concentration (2.75 g/L) (Press), gradual increase of salt concentration (Ramp), pulsed concentration with return to baseline (Pulse Return), and pulse with increased baseline (Pulse Ramp). Decomposition rate of leaf litter, Tipulid growth rate, and survival were monitored over the course of the study. Leaf litter mass lost was significantly greater in the two pulsed treatments than any other treatment, suggesting changing salt concentrations caused increased feeding in exposed Tipulids compared to other treatments. Despite greater consumption, Tipulid mass lost in the pulsed treatments did not vary significantly from other treatments. We hypothesize this result indicates increased osmoregulatory costs to tipulid larvae in the treatments with changing salt concentrations. Such costs may affect their ability to grow and reproduce, thus impacting the health of the streams as important shredder populations disappear.

### RP002 Evaluating the efficacy of native halophytes in the phytoextraction of sodium chloride (road salt) from contaminated soil

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Soil and freshwater salinization is a growing issue worldwide. Road salt, primarily sodium chloride, is a significant contributor to freshwater salinization in North America. When road salt is applied, it leaches into the roadside soil where it can accumulate, or leach further into nearby water systems. Increasing salinity poses a threat to both terrestrial and aquatic life, often creating inhabitable conditions. Salt tolerant plants, known as halophytes, thrive in saline soil, and select species are able to remove the salt from the soil, a process known as phytoextraction. Salts taken up from the soil are stored in the above ground biomass, and by harvesting this plant material salts can be removed from the soil before entering the water table. This study investigated the ability of four native halophytes: *Atriplex canescans, Atriplex patula, Atriplex hortensis,* and *Spartina pectinata* to remove sodium chloride from soil. Both field and greenhouse studies were completed in order to determine survivability in roadside areas, and $Na^+$ and $Cl^-$ extraction rates. This research aims to inform remediation and management practices for road salt contaminated soil in order to reduce the environmental impact of road salting. By intercepting the salt at the roadside, before it can leach further downstream, phytoremediation offers a proactive approach to mitigating freshwater salinization.

### RP003 Identifying and Evaluating Major Ion Toxicity of Coal Combustion Residual Landfill Effluents

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This project investigates the toxicological consequences of leachate from "dry" coal ash disposal on water quality and biota in receiving waters. The study includes quarterly whole effluent toxicity tests on discharge samples from three Maryland disposal facilities. Results indicate varying levels of chronic toxicity at all three locations. Priority metals and major ions have been measured and monitored over time and concurrently with bioassays. The wastewaters were found to be consistently high in total dissolved solids (TDS) and high conductivities were consistently observed, both indicative of elevated major ions. Traditional phase 1 toxicity identification evaluation (TIE) methods are not effective at addressing these issues. Models for predicting acute toxicity of a number of salt mixtures to *Ceriodaphnia dubia* have been developed, however, the thresholds at which chronic reductions in reproduction occur is still uncertain. Therefore, the purpose of the present study is to evaluate the toxicity associated with major ion imbalances using mock effluents and a weight-of-evidence approach in order to determine and confirm the primary causative ions. Calcium, chloride, magnesium, sodium, potassium, and sulfate were used to create a reconstituted mock effluent. A 7-day static renewal dilution series was completed using *Ceriodaphnia dubia* and threshold inhibition concentration values (IC25s) were compared between the reconstituted mock effluent and the wastewater. Subsequent toxicity tests were completed targeting single salts and salt mixtures in order to evaluate ion interactions and mechanisms of toxicity, as well as the ameliorative effects of ions in mixtures. Results of this work will advance our understanding of the relative risk of major ion imbalances in freshwater systems and can be used to refine treatment systems surrounding coal combustion residual facilities.

### RP005 The sensitivity of freshwater mussels to salt-laden winter bridge runoff; implications for mussel species at risk

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The increase in chloride (Cl) concentrations in North American surface waters over the past 30 years correlates with the increased application of de-icing salts. Freshwater salinization has implications for ecosystem health as chloride levels in urban streams frequently exceed those that are harmful to aquatic life. In winter, de-icing salt builds up in snowbanks on impermeable surfaces such as roads and bridges. When the temperature rises, the resulting salt-laden meltwater can be conveyed to aquatic habitats directly through bridge runoff or via overland flow. The early life stages of freshwater mussels are very sensitive to salt. This study focused on the Thames River (ON) watershed, the second most species-rich watershed for mussels in Canada. Two bridges in relative proximity to a four-lane expressway that span freshwater mussel species at risk habitat were targeted. The bridge's structures are such that runoff has different entry routes to the creeks below. Runoff from the Baptiste Creek bridge flows directly into the creek, whereas runoff from the McGregor Creek bridge travels through a tile drain before it enters the river. The concentration of chloride at the targeted sites was assessed throughout the year. During winter melt events, chloride and other contaminants were quantified in samples of snow from the bridges, bridge drain water, and creek surface water upstream and downstream of the targeted bridges. Mussel larvae (glochidia) were exposed to samples of field-collected meltwater in the lab using standard methods. Throughout the year, surface water in Baptiste Creek ranged from 9 to 89 mg Cl/L and McGregor Creek ranged from 39 to 323 mg Cl/L. In both cases, the maximum chloride concentrations corresponded to the colder season. Lab exposures with serial-diluted meltwater revealed 48-h EC50s for *Lampsilis fasciola* glochidia of 26.4% meltwater (2,764 mg Cl/L) at Baptiste Creek bridge (bridge deck drain sample = 8,250 mg Cl/L) and 43.8% meltwater (1,517 mg Cl/L) at McGregor Creek bridge (tile drain sample = 3,110 mg Cl/L). The distribution of freshwater mussels surrounding the targeted bridges is being examined to determine whether the toxicity observed in early life stage mussels exposed to winter road runoff in the lab translates into altered freshwater mussel distribution in the natural habitat.

**RP006 Toxic effects of "environmentally-friendly" deicing alternatives on Chironomus dilutus and a toxicologist's role in green marketing**

*M. Solan, Penn State Behrend; S. Nutile, Penn State Univ., The Behrend College / Biology*

Recent studies on the effects of increasing road salt pollution on aquatic organisms has incited development of new deicing formulations, many marketed as "environmentally friendly." With the ever-increasing development of "eco-friendly" products, the portion of the environment for which these products are aimed at protecting becomes increasingly blurred. As traditional deicing formulations consisting primarily of sodium chloride are well documented to significantly impact the health of aquatic ecosystems. As such, "environmentally-friendly" deicing alternatives may offer a solution to protecting human and aquatic health. Therefore, the goal of the current study was to determine the toxicity of traditional deicing formulations versus those deemed "environmentally friendly" using 10-d toxicity tests involving *Chironomus dilutus*. Second instar *C. dilutus* were exposed to either the reference toxicant (NaCl) at one of five concentrations (3.0, 4.5, 6.0, 7.5 or 9.0 g/L) or one of three alternative deicing formulations (calcium magnesium acetate, beet juice, urea) at concentrations determined via preliminary range finding tests. Lethal concentration 50 (LC50) values of each deicing formulation reveals the three "eco-friendly" deicing alternatives were equally or more toxic than traditional sodium chloride based deicing formulations to *C. dilutus*. Given these results, the role of toxicologists in validation of green marketing tactics is called into question. While these three formulations were not more protective of aquatic invertebrates, were they ever intended to be by the manufacturer? Developing a clearer definition of "eco-friendly" to ensure consumers are clear about the role they play in affecting and protecting aquatic environments upon which we all rely would aid in defining the role of green marketing in advancing scientific understanding.

## Assessment and Monitoring of Risks to Aquatic Environments from Short-Term (Episodic) Contaminant Exposures

**RP007 Exposure to untreated urban wastewaters alters growth and reproduction in *Daphnia magna***

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In November 2015, the repair of a sewage interceptor necessitated that nearly 5 billion liters of untreated wastewater (UWW) be diverted from a primary wastewater treatment plant (Montreal, QC, Canada) and discharged into the St. Lawrence River over the course of four days. These untreated UWWs originated from household, industrial, and hospital sources. The objective of this study was to investigate the effects of this planned UWW release on aquatic organisms in order to gain insight into the potential impacts of the increasing number of heavy rain events which may lead to unplanned UWW spills. Water samples were collected from four different sites along the St. Lawrence River during and four weeks after the release. Due to their high abundance as well as their importance in the St. Lawrence River food web, *Daphnia magna* were used as a model species. *Daphnia Magna* were experimentally exposed to the collected UWW from each site for 13 days and analyzed for a suite of biomarkers related to oxidative stress (i.e., catalase, superoxide dismutase, lipid peroxidation, and glutathione-*S*-transferase) and reproduction (chitinase). Results indicated that daphnid growth and reproduction were significantly impacted by the exposure to UWW when compared to the un-impacted reference site. Body size was smaller in *D. magna* exposed to UWW from one site and the number of neonates produced at that site was higher and decreased significantly after the event, suggesting long term reproductive impacts. These life-history-related effects were correlated with an increase in chitinase activity which is controlled by reproductive hormones and involved in growth. Overall, results suggest endocrine disruption potential of the UWW in these aquatic invertebrates. This study may contribute to a better understanding of the biological impacts of UWW to aquatic invertebrates.

**RP008 Short-Term in situ Monitoring Response of Diffusive Gradients in Thin Films in Marine Waters for Pulse Capture of Cd, Cu, Ni, Pb, and Zn**

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The ambient monitoring program, Project ENVironmental inVESTment (ENVVEST), being conducted for the Puget Sound Naval Shipyard & Intermediate Maintenance Facility (PSNS&IMF) in the Puget Sound receiving waters of Sinclair and Dyes Inlets, has conducted in situ aqueous metal bioavailability monitoring using diffusive gradients in thin film (DGT) passive samplers. In situ campaigns to record labile ($C_{DGT}$) Cd, Cu, Ni, Pb, and Zn have deployed DGTs in a manner that allows for short-term response characterization for deployment times ranging from 24 to 72 hours in low to moderate ambient conditions, and also overlaid in a manner that allows for capture of stormwater related fluctuations. The average relative percent difference (RPD) for 24 hour duplication, $n$=8, was 4±3, 15±18, 12±11, 135±62, and 57±44 for Cd, Cu, Ni, Pb, and Zn respectively; at ambient labile levels of 0.0368±0.00370, 0.302±0.104, 0.233±0.0267, 0.00640±0.0020, and 3.68±1.35µg $L^{-1}$. The average $C_{DGT}$ RPD when overlaying a 72 hour deployment onto consecutive 24 hour deployments, $n$=8, was 3±2, 18±14, 10±9, 45±20, and 79±36 for Cd, Cu, Ni, Pb, and Zn respectively. Metals with poor short-term performance, Pb and Zn, were affected by initial hyper-accumulation; which for Pb reflected values below the calculated $C_{DGT}$ MDL due to low ambient concentrations, and for Zn is theorized to be a naturally occurring result of proximity to industrial input sources. This initial dataset validates short-term DGT deployments for monitoring labile Cd, Cu, and Ni in seawater for the tested concentration range, and was followed by 8-72 hours deployments over a 2 week period, during both wet and dry seasons, at 7 stations within PSNS receiving waters.

## Improving Approaches to Assess Risks to Threatened and Endangered Species from Chemical Exposure

**RP009 Optimizing and evaluating a simplified toxicokinetic toxicodynamic model for predicting effects of pulsed exposure to carbaryl on fathead minnow**

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The effects of pulsed and time-variable exposures to chemicals may be under- or over-estimated with environmental risk assessment approaches based on standard, constant-exposure toxicity tests. However, fully calibrating models to predict the effects of pulsed exposures requires experimental data that are resource-intensive to gather and are not routinely provided for the EPA's pesticide registration process. Previous researchers have suggested that standard 96-h acute and 30-d early lifestage toxicity test data may be used to parameterize a simplified toxicokinetic, toxicodynamic (TKTD) model, resulting in predictive power equivalent to a full model. Our team is developing an efficient protocol using R to evaluate the characteristics of toxicological datasets that result in successful parameter estimation for this simplified model, and to select the toxicological data that results in models with optimal predictive power. We demonstrate our methods and results using the effects of carbaryl on fathead minnow survival as an example, with datasets derived from both standard and non-standard toxicity test endpoints, as well as literature-estimated parameters. We also discuss the possibility of accounting for size-dependent sensitivity and sublethal endpoints within these models, developing a generalized model for other species/chemical combinations, and using pulsed exposure effects model outputs to inform population models. This work is a part of the fish population model development at EPA-MED, presented elsewhere during this session.

**RP010 Extrapolating from Laboratory-based Aquatic Toxicity Tests to Fish Populations: Data from Diazinon and Carbaryl Exposures**

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The United States Environmental Protection Agency (USEPA) assesses the risk of pesticides that are registered under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) to the environment and non-target wildlife and fish. A fundamental challenge in this process is the extrapolation from laboratory-based aquatic toxicity testing, which collects data on the individual fish, to population-level impacts. Currently, we are developing a structured population modeling (called the Fish Translator) that is intended to bridge the gap between effects observed in standardized aquatic toxicity tests often submitted during the FIFRA pesticide registration process, and potential outcomes of pesticide exposure to fish populations. As part of this research effort, laboratory tests on fish exposed to pesticides have been conducted to provide data on the relationship between growth and reproductive output (*i.e.* initiation of spawning, duration of spawning, and fecundity) to help parameterize the Fish Translator model. Specifically, Japanese medaka (*Oryzias latipes)* were exposed to either diazinon (an acetylcholinesterase inhibitor), reared at reduced food rations, or both to dis-entangled the reproductive and growth effects of a pesticide and the relationships between exposure, growth, and reproduction. Additionally, a standard Fish Early Life Stage Test (OCSPP 850.1400) with carbaryl (another acetylcholinesterase inhibitor) was conducted, exposing fathead minnows (*Pimephales promelas*) which were then reared without exposure to reproductive competence. The goal is to link adverse outcomes of an early life exposure (data often submitted under FIFRA) to adverse reproductive outcomes later in life that, in turn, are applicable to populations and population modeling. The effects observed in the laboratory of these two pesticides on the reproductive output of either Japanese medaka or fathead minnow were used in the Fish Translator to predictive population-level impacts on fish species with similar life history parameters.

**RP011 Assessing the Population Impacts of Pesticides on Delta Smelt**

*M. Etterson, N. Pollesch, S. Kadlec, K. Flynn, USEPA / ORD / NHEERL / Mid-Continent Ecology Division*

Under section 7 of the US Endangered species act,USEPA is responsible for ensuring that its actions, including pesticide registrations, do not place federally listed species at jeopardy. Here we evaluate the utility of a new USEPA fish population model, protoyped for Fathead Minnow (*Pimephales promelas*) when parameterized and applied to Delta Smelt (*Hypomesus transpacificus*), a federally listed endangered species. The model is a size-structured integral projection model that can incorporate chemical and non-chemical stressors to estimate effects on population trajectories. Here we present how this modeling framework can be adapted for the life history of the delta smelt, where different life-stages occupy spatially distinct areas, reproduction is semelparous, and pesticide exposure occurs in pulses.

**RP012 Implementing a flexible approach to address the risk of pesticides to threatened and endangered species**

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Pesticides are tools commonly used to manage habitat and to control unwanted pests, but their use comes with inherent risks. Management of the risks to listed species is complicated by the fact that listed species are widely distributed into a variety of habitats throughout the United States and site-specific factors highly influence risk. For example, the presence of riparian buffers can mitigate risk to aquatic species by improving water quality and reducing pesticide loading into aquatic habitats. A "one size fits all" approach to managing risk is not always necessary or desired. In a recent Biological Opinion, the National Marine Fisheries Service (NMFS) proposed the option of managing risk using a "flexible toolbox approach" conceptually similar to that described in the proceedings from the recent SETAC MAgPIE workshops. This presentation will outline the NMFS approach which essentially awards risk reduction points depending on site-specific characteristics, application-specific methodology, and the use of drift reduction technologies. This approach encourages partnerships, recognizes land managers and pesticide applicators that participate in approved conservation activities and pesticide stewardship plans, and provides site-specific flexibility in reducing pesticide risk at a nationwide scale.

**RP013 Save Two Birds with One Stone: Protect Listed Species & Ecosystem Services in Geographically Isolated Wetlands Using Community-Level Protection Goals**

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Geographically Isolated Wetlands (GIWs) are unique geological features that provide habitat for a number of endemic and endangered and threatened animal and plant species, as well as providing a number of ecosystem services (ES). GIWs are threatened by a range of chemical contaminants that vary based on region and assessing potential impacts to the fragile communities they embody is a challenge. We propose an approach that develops community-level protection goals for GIWs that include both listed species and taxa that drive ES for ecological risk assessments (ERAs). Community-level protection goals are derived by weighting the prioritization of listed species followed by taxa providing ES, allowing a single risk ERA to establish protection levels for multiple species in one process. We identify chemical hazards specific to each type of GIW within the US and derive protection goals for each based on their community of endemic and listed species and ES components. We discuss exposure pathways for GIWs in general and examples of exposure and effects models that can be used to achieve the protection goals. We also explore weight of evidence approaches that aid in establishing hazard concentrations based on contaminant type, exposure pathways, and available effects data. We demonstrate the process using a case study of vernal pools in California's central valley.

## Current-Use Pesticides: Exposure and Effects on Non-Target Organisms and Ecosystems

**RP014 Pesticide distribution trends in the Canadian Prairie Pothole Region**

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Pesticides often occur in wetland ecosystems as complex mixtures with strong seasonal and geographic distribution. Frequently detected pesticides in the Canadian Prairie Pothole Region (PPR) are likely related to the high intensity use of these compounds and to the crop type surrounding the wetlands. Pesticide use estimates that link these two variables will be presented in order to identify the main factors that drive pesticide spatial distribution and prioritize areas for future large-scale, targeted pesticide sampling in Prairie wetlands. Using a GIS-based modeling, we estimated the spatial distribution of key pesticides in the PPR. The use distribution was computed by intersecting percentage land cover with application rates for each crop and province. The application rates were derived from a large commercial survey conducted in 2015. There were 12 insecticides, 35 fungicides and 56 herbicides used in the PPR in 2015. All insecticides were used for the insecticide distribution model, whereas for herbicide and fungicide models compounds were prioritized based on mass, ecotoxicological relevance and fate data. Pesticide distribution models were able to identify specific use hot-spots in the PPR, which were primarily related to canola and wheat crops. The province of Saskatchewan had the highest application rates for all three groups of pesticides (insecticides, herbicides and fungicides). For example, use of the insecticide chlorpyrifos was 22-fold higher in Saskatchewan compared to the other provinces of Alberta and Manitoba. Based on these results and the distribution of water in the Prairies, two monitoring campaigns were designed for implementation in 2017 and 2018 with the ultimate goal of estimating the ecological risk from pesticides in PPR wetlands.

**RP015 Neonicotinoids and phenylpyrazoles, metabolites and pesticide synergists in surface waters and sediment samples of British Columbia, Canada**

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Neonicotinoid and phenylpyrazole insecticides have received significant attention due to suspected effects on non-target aquatic and terrestrial insects. Current analytical approaches for these analytes do not typically measure insecticide metabolites, which are also toxic, and may not include pesticide synergists, whose presence can increase the toxicity of the insecticides. In the present work, we developed an isotope dilution ultra high-performance liquid chromatography (UHPLC) tandem mass spectrometric method for the quantitative measurement of 8 neonicotinoids and 6 neonicotinoid metabolites, the phenylpyrazole fipronil and 4 of its degradation products, and 2 commonly used pesticide synergists. The method used ultrasonic extraction followed by solid phase clean-up for solid samples and solid phase extraction and cleanup for aqueous samples. Sample extracts were analyzed by UHPLC (+/-)ESI MS/MS. The method's detection limits ranged from 1.25 to 2.5 ng/L for the insecticides, 1.25 to 40 ng/L for the metabolites and 1.25 to 10 ng/L for the pesticide synergists in aqueous samples. Detection limits for sediment samples ranged from 0.1 to 0.2 ng/g, 0.2 to 3.2 ng/g, and 0.1 to 0.8 ng/g wet weight respectively. In replicate spike recovery experiments, n=5, recovery for the phenylpyrazole fipronil and its metabolites ranged 97-100% (RSD 0.9 – 3.1%) in aqueous and 99-106% (RSD 0.9-8%) in solid samples. Similarly, recovery for neonicotinoids and their metabolites ranged 69-125% (RSD 2-11%) in aqueous samples and 55-120% (RSD 2-24%) in solid samples. Performance measures were satisfactory for all targets except imidacloprid urea where some low recoveries were observed in both aqueous and solid samples. The developed method was applied for analysis of paired water and sediment samples from three diverse sites in British Columbia, Canada. Sampling locations included five sites near agricultural land use, five sites near urban land use and two reference sites. The partitioning behavior of these contaminants will be discussed. This data contributes to our understanding of the occurrence, fate and behaviour of some of the most widely used insecticides in the world.

**RP016 Imidacloprid use and contamination in surface waters in the Central Coast and Imperial Valley, California**

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Imidacloprid is an insecticide that has been regularly monitored in California's Salinas, Santa Maria, and Imperial valleys since 2010 by the California Department of Pesticide Regulation's Surface Water Protection Program (SWPP). The three regions represent agricultural croplands dominated by row crops. Typically, irrigation practices on row crops tend to generate higher pesticide runoff that has the potential to pollute aquatic environments adjacent to pesticide-treated fields. SWPP monitoring events occurred during the dry season in each region to coincide with the period of highest imidacloprid use during the year. Imidacloprid use exhibits distinct regional patterns. Crops with the highest use are lettuce in the Salinas Valley, broccoli in the Santa Maria Valley, and sugar beet and lettuce in the Imperial Valley. Imidacloprid was detected in 85% of 380 samples collected from 30 sites during the dry season in 2010–2016 making it the most detected compound among all the pesticides monitored by SWPP. When comparing reported concentrations to the recently revised USEPA imidacloprid aquatic life benchmarks, 52% of the samples had imidacloprid concentrations that exceeded the acute benchmark of 0.385 μg/L, and all the samples had concentrations that exceeded the chronic benchmark of 0.01 μg/L. Among the three regions, the watersheds in Santa Maria showed significantly higher detection frequencies and concentrations, followed by those in Salinas and Imperial. Site-specific analyses will be conducted at the catchment and watershed levels to elucidate the high surface water concentrations associated with imidacloprid use patterns.

**RP017 Ultra-trace analysis of pesticides by HPLC-MS/MS: A pilot survey of drinking water, fruit, and vegetables in Quebec, Canada**

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The extensive use of certain pesticides and their relative persistence goes on par with the presence of residue levels in drinking water and/or agricultural products available to consumers (including food commodities), with possible implications for human exposure. In view of these findings, quality standards have been proposed for drinking water; maximum residue limits (MRLs) of pesticides have also been established for foodstuff available to consumers. This requires the implementation of sensitive and robust analytical methods for the ultra-trace quantification of these compounds, to determine whether the different samples are compliant to guidelines or MRLs. A commonly used method for polar pesticides in water samples relies on off-line solid phase extraction (SPE) of a large sample volume followed by liquid chromatography tandem mass spectrometry. In the case of food analysis, the QuEChERS method is gaining popularity due to its wide range of application for polar compounds and cost-effectiveness. Within this context, the overarching aim of this work was to propose fast and robust analytical methods to determine multi-class pesticides in drinking water and food commodities. For water samples, we set out to develop a fully automated on-line SPE method coupled to ultra-high-performance liquid chromatography tandem mass spectrometry. The method is rapid (8 min per sample), with limits of detection in the range of 0.1–5 ng $L^{-1}$. Factors that could affect the whole-method accuracy were examined (e.g., filtration artifacts, time-dependent storage stability, and matrix effects). For fruit and vegetables, we optimized a modified dSPE QuEChERS method, e.g., by varying extraction salts and contact time as well as clean-up sorbents. The modified dSPE method showed LODs between 0.05 $ng \cdot g^{-1}$ and 2 $ng \cdot g^{-1}$. The robustness of the method was demonstrated via quality control experiments to ensure the lack of matrix effects in along the analytical sequence. The validated methods were applied to real samples including tap water samples covering 50 cities in the Quebec province (Canada), and 133 samples of lettuce, apple, grapes, and tomatoes purchased across local markets. The results indicate the occurrence of thiamethoxam, clothianidin and imidacloprid in the water samples and four commodities. Dimethoate and triethylphosphorothioate were also occasionally observed. Nevertheless, the concentrations remained below the MRLs established.

**RP018 Pesticide Encapsulation at the Nano Scale Drives Changes to the Hydrophobic Partitioning and Toxicity of an Active Ingredient**

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The multibillion dollar pesticide industry promotes innovative technologies that enhance existing products. Given the costs associated with designing novel active ingredients, many new formulations focus on the use of other ingredients to modify existing formulations. Among these developing products are encapsulated pesticides, which shield the active ingredients from immediate interactions with their surrounding chemical environment and offer a variety of enhanced features including controlled release and improved efficacy. Despite the presence of nano-sized capsules in commercial pesticide formulations, the analytical and toxicological implications of encapsulation are uncertain. Moreover, there is limited information regarding the relevance of capsule size on capsule-based effects. To explore this issue quantitatively, we fractionated the capsules of a commercially available encapsulated insecticide (γ-cyhalothrin active ingredient) into two size ranges: a large fraction (LF), with an average hydrodynamic diameter (HDD) of 758 nm, and a small fraction (SF), with an average HDD of 449 nm. We developed a novel extraction method capable of comparing the partitioning behavior of encapsulated γ-cyhalothrin, an extremely hydrophobic insecticide, with freely suspended γ-cyhalothrin. Both encapsulated fractions showed a time-dependent inhibition of γ-cyhalothrin hydrophobic interaction for up to 48 hours. To assess any capsule effects on γ-cyhalothrin toxicity, we conducted an acute immobilization test with a freshwater macroinvertebrate (*Ceriodaphnia dubia*), normalizing treatment concentration of the active ingredient. The SF was significantly more toxic than both the LF and the free γ-cyhalothrin treatment ($EC_{50}$ = 0.18 μg/L, 0.57 μg/L, and 0.65 μg/L respectively), while the toxicity of the LF was similar to the free γ-cyhalothrin. Together, these findings highlight that encapsulation of γ-cyhalothrin mitigates hydrophobic partitioning in a time-dependent manner and influences toxicity in a size-dependent manner. Recognizing the analytical and toxicological nuances of various nano-sized capsules can contribute to innovation in pesticide formulations and may lead to more comprehensive pesticide regulation.

**RP019 Composition and function of nicotinic acetylcholine receptors in *Chironomus dilutus* exposed to neonicotinoid mixtures**

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Neonicotinoids and their mixtures are routinely detected in global freshwater environments and can exert greater than additive toxicity to non-target invertebrates. Previous studies have characterized the toxicities of select neonicotinoids and their mixtures using *Chironomus dilutus* as a representative aquatic test species. However, toxicological effects tend to vary depending on exposure duration and condition, mixture composition, and endpoint of concern. One hypothesis for these diverse effects is that the function and molecular composition of the nicotinic acetylcholine receptor (nAChR), the main molecular target of neonicotinoids, varies in response to exposure to different neonicotinoids and their mixtures. Therefore, this research aims to characterize the effects of select neonicotinoids (imidacloprid, clothianidin, and thiamethoxam) and their mixtures on the composition and function of nAChR in larval and adult *C. dilutus*. Lab-reared organisms were exposed to either single compounds or binary mixtures at equivalent dose-levels (28 d $\Sigma$ $EC_{12.5}$ for *C. dilutus* emergence) for 28 days. Larvae were sampled after 10 d of exposure, and emerging adults were collected until the cessation of the study. Putative *C. dilutus* nAChR subunits were identified by searching non-redundant nucleotide databases using nAChR subunits of *Anopheles gambaie* and *Drosophila melanogaster* as references. Quantitative real-time RT-PCR was used to assess nAChR transcript abundances among neonicotinoid/mixture treated larvae and adults. Radioligand binding assays were used to assess the effects of neonicotinoids/mixtures on functional expression by evaluating the affinity of expressed nAChR proteins for select neonicotinoid agonists (IMI, CLO, TMX). Results obtained will be compared to single compound/mixture toxicity models to determine if changes in nAChR composition or functional expression correlate with toxicological responses in *C. dilutus*. This will ultimately enhance our understanding of chronic neonicotinoid mixture effects on non-target aquatic insects, helping improve risk assessment practices for this class of compounds.

**RP020 Molecular effects of imidacloprid to *Chironomus dilutus* by de novo transcriptome**

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Neonicotinoids are one of the most widely used systemic insecticides all over the world. Their wide distribution in the environment has posed a threat to non-target species (Hladik et al. 2018), ultimately causing concern about risk to biodiversity and ecological function (Chagnon et al. 2015). However, molecular mechanisms underlying these effects has not been well characterized yet. This study evaluated adverse effects of a neonicotinoid, imidacloprid, to a benthic invertebrate, *Chironomus dilutus,* and explored the probable toxicity pathways by *de novo* transcriptome. Acute and sublethal toxicity of imidacloprid to *C. dilutus* were detected. With the increasing concentration of imidacloprid, midge larva lost burrowing capacity, and could not move normally by the s-shaped curve, lead to overexcitation, and finally paralysis to death. Imidacloprid produced neurotoxicity, oxidative stress, endocrine disruption and immunotoxicity by *de novo* transcriptome analysis. Molecular toxicity pathways disrupted by imidacloprid at different concentrations were evaluated by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses. By GO enrichment analysis, sodium ion transport and mitochondrial electron transport were easily affected, suggesting possible damages on neural system and energy metabolism. Besides, chitin binding was enriched, which was related with endocrine effect. By KEGG pathways analysis, peroxisome proliferators-activated receptors (PPARs), calcium, glucagon, fatty acid degradation, apoptosis signaling pathway were enriched. These pathways were related to oxidative stress and disfunction on endocrine and nerve systems. Our study offered better understanding on molecular mechanism of imidacloprid toxicity to *C. dilutus* and helped evaluating the risk of the exposure of aquatic species to neonicotinoids at environmentally relevant concentrations. Reference Chagnon, M., D. Kreutzweiser, E. A. Mitchell, C. A. Morrissey, D. A. Noome and J. P. Van der Sluijs., 2015. Risks of large-scale use of systemic insecticides to ecosystem functioning and services. Environ. Sci. Pollut. 22, 119-134. Hladik, M. L., A. R. Main and D. Goulson., 2018. Environmental risks and challenges associated with neonicotinoid insecticides. Environ.Sci. Technol. 52, 3329-3335.

**RP021 Genotoxicity Assessment of Imidacloprid on Root Meristem of *Allium cepa***

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Myriads of literatures have critically examined the toxicity of Imidacloprid as well as its possible ecological effects. However, few studies have examined its toxicity at the genetic level. In this study, the effects of Imidacloprid on *Allium cepa*, following the exposure of *Allium cepa* to different concentrations of this insecticide was evaluated. After the 96 hours of exposure, the highest growth inhibition was observed in the onions treated with 1.0mol/L of Imidacloprid measuring average length of 9mm, while the least inhibition of 13.8mm was in the control. The results demonstrated that the concentrations tested induced chromosomal alterations and also had an effect on onion growth. The sample tested with 0.4mol/L concentration of test substance recorded the highest number of aberrant cells with a total number of 14 aberrant cells. Concentrations higher than 0.4mol/L showed lesser number of dividing cells, hence lower observation of aberrant cells. Therefore, Imidacloprid in these concentrations was genotoxic to the tested organisms. Considering its genotoxic nature, information concerning the extent of its genotoxicity should be disseminated.

**RP022 Imidacloprid-containing pesticides disrupt C. elegans germline development**

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Neonicotinoids are a class of pesticide that has been under investigation by the Environmental Protection Agency (EPA) in recent years. A major concern is a possible role they may play in colony collapse disorder in honey bees, wherein neonicotinoids disrupt both their development and behavior. One example of a neonicotinoid is imidacloprid, which is an active ingredient in pesticides which are used in the agriculture industry and in pesticides available to the general public for use in lawn and garden care. Here, the effects of an imidacloprid-containing pesticide on the development of *C. elegans* was investigated. *C. elegans* is a model organism which has been used in assessing the effects of chemicals including BPA. The assessment was conducted on three different strains of *C. elegans,* wild-type, *bus-17* which has a defective cuticle and in the transgenic strain *ced-1*::GFP. The imidacloprid containing pesticide used in the investigation is one which is available to the general public and which contains imidacloprid at a concentration of 1.47%. Specifically, we investigated the effect of imidacloprid containing pesticides on the *C. elegans* germ line, a population of cells that is of particular importance as exposure to toxins can affect subsequent generations. To determine the effects of imidacloprid on germ cell development, we scored apoptotic nuclei and found that imidacloprid caused a significant increase in the number of corpses as a result of exposure to the imidacloprid containing pesticide. Accordingly, we also discovered a reduction in fertility in the offspring, as determined by brood size analysis with which exposure to the imidacloprid containing pesticide was found to lead to a significant decrease in brood size. We are currently exploring the nature of these defects, which strongly indicate that imidacloprid negatively impacts development and reduces gamete quality. Our data support findings that neonicotinoids cause widespread harm to multiple species and raise concern about their safety to humans as it shows elevated levels of apoptosis in all three strains of *C. elegans* and a reduction in fertility in response to exposure to the imidacloprid-containing pesticide.

**RP023 Long-term exposures of neonicotinoid insecticides to earthworms**

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Concerns about the effects of neonicotinoid pesticides to non-target organisms have been mounting based on evidence of impacts on pollinators. Soil invertebrates such as earthworms are also susceptible to neonicotinoid exposure due to potentially extensive neonicotinoid residence times in soil and the likelihood of multiple applications per growing season. Standardized testing has contributed the development of baseline toxicological data for many of the standard species, including the earthworm *Eisenia andrei*. While sensitivity has been well-documented, measured environmental concentrations of neonicotinoid insecticides in soil are significantly lower than those known to cause adverse reproductive effects in this species. In these studies, we hope to account for both the potentially long duration of neonicotinoid residence in agricultural soils and low concentrations at which they have been detected by exposing one parental and three successive filial generations of *E. andrei* to single applications of the neonicotinoids Thiamethoxam and Clothianidin in an Ontario agricultural soil.

**RP024 Pyrethroid concentrations toxic to *Hyalella azteca* in an urban creek diluted to non-toxic levels by municipal wastewater discharge**

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Waterways in California's Central Valley have been determined to be impaired due to toxicity from pyrethroids. This has spurred California's Regional Water Quality Control Boards to promulgate regulations to address discharge of pyrethroids from agriculture, urban stormwater, and municipal wastewater sources. Many municipal wastewater treatment plants (WWTPs) in California utilize tertiary-level treatment. Often this high level of treatment is capable of meeting water quality objectives/criteria end-of-pipe, which is desirable when the receiving water is dominated by the effluent discharge, even though there can be other pollutant sources in the watershed. We conducted a year-long study to evaluate pyrethroid levels and toxicity in Central Valley creeks receiving effluent discharges from two California tertiary WWTPs. For both sites, water samples were collected from the discharge, upstream, and downstream creek stations for pyrethroid chemical analysis (detection limits as low as 5 pg/L). Effluent and creek samples for one site were also tested for acute toxicity with the crustacean, *Hyalella azteca*, a species sensitive to pyrethroid toxicity. Results demonstrate that tertiary-level treatment of municipal wastewater provides high pyrethroid removal efficiencies and that pyrethroid levels in the treated effluent were low and not toxic to *H. azteca*. The highest pyrethroid concentrations and the sole instance of toxicity were observed at an upstream creek station. This occurred during a first-flush rain event where the predominant land use is urban-residential. Dilution of upstream creek water by the WWTP's effluent discharge resulted in non-toxic conditions downstream during the same first-flush rain event. The study demonstrates that discharge of tertiary-treated WWTP effluent to surface waterbodies, although often considered a stressor itself, can benefit downstream water quality through high constituent removal efficiencies which can facilitate diluting upstream non-point sources of pollutants.

**RP025 Where is the biology in terrestrial vertebrate pesticide risk assessments?**

*C. Habig, Compliance Services International*

The EPA evaluates potential risks to birds and mammals using its T-REX model. T-REX is an intentionally conservative screening-level spreadsheet model with a low number of user inputs that estimates potential exposure and risks to birds and mammals through ingestion of feed items containing pesticide residues. The model includes five generic categories of feed items – short grass, tall grass, broadleaf plants, arthropods, and seeds, pods, and fruits. Upper-end pesticide residues are estimated for each of these feed item categories based on the Fletcher et al modifications to the Hoerger and Kenaga nomogram. These residue estimates are paired with toxicity values for the compound being evaluated, and the model then calculates acute and chronic risk quotients (RQs). To pass, acute RQs for non-endangered species need to be < 0.5, while acute RQs for endangered species need to be < 0.1. Chronic (or longer-term) RQs need to be < 1.0 to pass. If a product fails the T-REX assessment, what are the options for refinement of the assessment? Options for quantitative refinement are often minimal, and typically focus on evaluating possible options for changes to the use pattern or use scenario. These changes may include changes to the maximum application rate, the number of applications in a season, the interval between applications, and, if data are available, the foliar dissipation rate. However, qualitative refinements that focus on biological attributes of key species of concern are also feasible, but are often overlooked. These types of considerations include factors such as more detailed analysis of the types of food ingested (preferred foods), behavioral aspects such as how animals primarily forage for food (e.g., a ground forager, foraging on flying insects, foraging on soil invertebrates), as well as a species' preferred habitat. These considerations are particularly useful when evaluating potential effects on endangered species that may be found near agricultural use sites. Examples of the impacts of these more in-depth considerations will be provided for a) a product that is soil incorporated; and b) species that forage on flying insects around treated areas.

**RP026 Wild bees and pesticides: Reported applications compared to detections in multiple matrices in an intensively managed agricultural landscape**

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To better understand the exposure of wild bees to pesticides in an agricultural landscape, samples were collected from fields in northern California. Hedgerows are known to provide habitat for wild bees, but these bees may also be exposed to pesticides from nearby agricultural fields. The current study targeted eight hedgerow sites located in an intensively managed agricultural landscape that includes almonds, (wine) grapes, rice, tomatoes, and walnuts. In addition to collecting both wild bees and honey bees, soil, flowers, and silicone passive sampling devices (PSD; staked near the hedgerows to sample the air) were also included. Sampling was conducted from April to June 2016, to coincide with peak bloom and bee activity. Samples were analyzed for >150 pesticides and degradates using both gas and liquid chromatography-tandem mass spectrometry. Overall, 38 pesticides were detected in all matrices (10 insecticides and degradates, 12 fungicides, 15 herbicides and degradates, and 1 plant growth regulator). The number and type of pesticides detected varied by matrix; 24 compounds were detected in the PSDs, 24 in soil, 15 in flowers, 18 in wild bees, and 10 in honey bees. Pesticide application information for the locations and sampling time frames were compared to the detections. These results can help determine which matrices are best at estimating wild bee pesticide exposure and which pesticides should be targeted for future effects work.

**RP027 Organosilicon adjuvants in pollen: Initial results from a 2018 field survey**

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Adjuvants are chemicals that are added to enhance the performance of the active ingredient(s) in pesticide products. Trisiloxane surfactants (TSS) are an important class of adjuvants used in various pesticide spray applications. Commercial TSS products are oligomer mixtures typically consisting of a hydrophobic trisiloxane backbone with various hydrophilic side chains composed of ethylene and propylene oxide (EO/PO) units capped with acetoxy, hydroxy, and methoxy end groups. These TSS surfactants have been identified in pollen, beeswax, and honey, and recent studies report that TSS surfactants can impair honey bee learning and interact synergistically with viral infections in honey bee larvae. As part of a larger study, designed to examine the impact of TSS on honeybees, we examined the extent of TSS contamination in pollen samples collected by various beekeepers from hives located in California and Kentucky during February and March 2018. Pollen samples were extracted with acetonitrile and analyzed by high performance liquid chromatography coupled to a triple-quadrupole mass spectrometer (LCMS-QQQ). Limits of quantitation (LOQ) for the acetoxy, hydroxy, and methoxy-capped trisiloxanes oligomers were 1.18-10.58ng/g, 0.18-7.05ng/g, and 0.29-2.94ng/g, respectively. In the 27 samples analyzed to date, hydroxy-capped trisiloxanes were found in over half the samples with concentrations in the low ng/g levels for the sum of all hydroxy oligomers (oligomers n = 6-8 were the most commonly found). Little to no acetoxy and methoxy-capped trisiloxanes have been found above the LOQs so far. However, it should be noted that recoveries of TSS added to clean pollen sent to the field and back were much lower than freshly spiked pollen. Additional extraction and clean up methods are being evaluated. A preliminary pesticide screening analysis for over 60 pesticides was also performed on each sample using gas chromatography-mass spectrometry. To date, three fungicides have been tentatively identified: cyprodinil, pyrimethanil, and propiconazole.

**RP028 Estimating Neonicotinoid Residues in Pollinator-Attractive Habitat by LC-MS/MS Analysis**

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Recent research has suggested that neonicotinoid insecticides applied to crops can be detected in adjacent pollinator-attractive habitats and pollen collected by honey bees. Honey bees, native bees, and monarch butterfly larvae could be exposed to neonicotinoids through contact with or ingestion of contaminated pollen, nectar, and milkweed leaves. Although these studies indicate that neonicotinoids can be detected in pollinator-attractive habitats, the magnitude and extent of potential adverse effects to honey bees, native bees and monarch larvae is an active area of research. In this study, we developed and validated an innovative method to simultaneously evaluate concentrations of clothianidin, imidacloprid, and thiamethoxam, and two imidacloprid metabolites (5-hydroxy imidacloprid and imidacloprid olefin) in plant foliage. The results indicate that we have acceptable ranges for the recovery, low and high quality control (between 75% and 110%), while the testing for a matrix effect indicates minimal ion suppression or ion enhancement for all analytes. The calibration curves were linear over the concentration ranges with $r^2$ at > 0.998.The data obtained established a validated, single extraction and LC-MS/MS analytical method for quantifying neonicotinoid concentrations to a method detection limit of 0.04 to 0.3 ng/g plant material. This method is comparable, or in some cases better than, existing methods that require separate extraction and/or LC-MS/MS methods. We are using the method to analyze leaf and pollen samples collected in 2017 and 2018 from habitat patches adjacent to corn and soybean fields planted with neonicotinoid-treated seeds. This data will provide insights on spatio-temporal variability of neonicotinoid concentrations in Midwestern agroecosystems and help inform pollinator risk assessments.

**RP029 A Novel System to Study the Effects of Agrochemicals on Honey Bee Queen Health and Fecundity**

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As the sole producer of fertilized eggs in a colony, honey bee queen health and reproduction are essential to the longevity of a colony. Recent research has demonstrated negative effects of agrochemicals on colony reproduction, including decreased colony expansion, queen failure and replacement, and decreased queen egg laying. This suggests that agrochemicals can have negative effects on queens as well as workers. However, it is much more difficult to quantify the performance of queens relative to workers in the field, and there are no laboratory assays for queen performance. This presentation will describe a new system developed to study the effects of numerous stressors on honey bee queen egg laying under tightly controlled, laboratory conditions using custom designed Queen Monitoring Cages (QMCs), yielding a quantitative endpoint. The results of the first experiment in this system examining the effects of imidacloprid administered in worker diet on queen egg laying will be described, and the results will be compared to previous work in full sized colonies exposed to dietary imidacloprid. Additionally, queen retinue behavior will be quantitatively assessed in workers following chronic exposure to imidacloprid to determine if imidacloprid affects this behavior. Taken together, the results of these experiments will provide insight into the sociotoxicology of agrochemicals in honey bee colonies and the consequences to queen fecundity. Furthermore, these experiments will provide a framework that can be used to study other potential stressors and their impacts on colony health, including pathogens, parasites, poor nutrition, and interactions between them in a more controlled context.

**RP030 Effects of neonicotinoid insecticides on hummingbirds**

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Neonicotinoids are neurotoxic systemic insecticides that have become the most widely used group of insecticides worldwide. These compounds affect the nervous system, interfering with the transmission of nerve impulses and impairing vital physiological processes. There is a growing concern about their environmental impacts, particularly linked to bee colony collapse disorder and drastic reductions in insect populations. While the loss of pollination services provided by bees to ecosystems and agriculture has been widely studied, the neonicotinoid effects in other pollinators such as hummingbirds haven't been assessed yet. Hummingbirds in particular present a great risk of exposure to pesticides due to their high daily nectar intake, making them also more vulnerable to the adverse effects of these toxic substances. We examined the sublethal toxic effects of the neonicotinoid imidacloprid in captive Ruby-throated hummingbirds (*Archilochus colubris*). Using a multibiomarker approach we evaluated biochemical, physiological and behavioral impairments. Two doses were tested, a high dose based on previous toxicity assessments in similar size species and a low dose calculated from the potential daily consumption of imidacloprid by hummingbirds feeding in blueberry crops. To determine cellular neurotoxic effects, Cholinesterase activity and oxidative stress response were measured. As physiological response Resting Metabolic Rate and immune function were examined. Likewise, the toxicokinetics of the insecticide were assessed through urine analyses. This is the first study assessing the effect of pesticides in hummingbirds, our results will provide insights of pesticide exposure as a potential threat for populations of these species and will help management agencies improve regulation.

**RP031 Further developments of a passerine model of subacute neonicotinoid toxicity using zebra finches**

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Neonicotinoid insecticides are nicotinic acetylcholine receptor (nAchR) agonists commonly used in agriculture as seed coatings. While these compounds were developed to be insect nAchR specific, population declines of wild birds have been attributed to these chemicals, and behavioral, reproductive, and lethal effects have been experimentally documented in laboratory-exposed birds. Our goal is to develop a model of subacute neonicotinoid (clothianidin (CTD)) toxicity in an altricial passerine (zebra finches (*Taeniopygia guttata*)). In a series of studies differing mainly by exposure duration and medium, we first determined an acute effects concentration of CTD by treating fasted birds with coated millet seed (CTD treatments range, 15 – 1000 mg/kg bw). Above 125 mg/kg bw (males) and > 60 mg/kg bw (females), we observed strong refusal of coated seed. At these treatment levels, birds consumed approximately 2.0 – 0.26 mg CTD / bird. Within 2 hr after treatment with 125 mg/kg we observed inactivity, fluffed feathers, respiratory difficulty, and, in some cases, inability to fly. Based on these results, we estimate the NOAEL and the LOAEL for our study to be 15 and 30 mg/kg bw, respectively. We next exposed birds for 7 days to CTD in drinking water at nominal doses of 8.6, 4.3, 2.2, and 0 mg/kg (cumulative 60, 30, 15, and 0 mg/kg CTD). Clinical signs of toxicity were not observed in any bird. Lastly, birds were gavaged daily for 7 days with 18 mg/kg bw dissolved in acetone and mixed with vegetable oil. Clinical signs of toxicity were observed beginning after the fourth exposure day and were similar to signs observed in acute (125 mg/kg) CTD dosing. The birds were video recorded at the same time each afternoon and activity was quantified. Generally, behavior returned to normal each day 6-8 h after dosing with CTD. At 14 days following either the single acute dose or the final dose in the subacute study, all birds were euthanized and histopathology was performed on liver tissue and brain acetylcholinesterase (AChE) level determined. In birds dosed for 7 days, lesions were not observed in liver tissue and the AChE activity was normal. Our work provides further information on experimental exposure strategies for a model passerine and helps to set the range of CTD that might be used in a subacute or chronic toxicity trial in zebra finches.

**RP032 Use of dermal absorption data for estimating avian dermal LD50s and dermal exposure in higher-tier risk assessment models for pesticides**

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Estimating avian exposure via a dermal pathway is essential for higher-tier ecological risk assessments (ERA). However, dermal exposure and toxicity data for birds are seldom available. The USEPA's higher-tier Terrestrial Investigation Model (TIM) uses a method to estimate avian dermal LD50 values and dermal exposure that for some compounds results in estimates of high dermal exposure and low dermal LD50 values. This pattern is likely attributable to using organophosphate and carbamate toxicity data to develop the oral-dermal relationship (i.e., an estimated dermal LD50 is necessary to generate a dermal route equivalency factor that normalizes potency relative to oral toxicity within TIM's dermal pathway dose equation). However, use of these relationships may introduce uncertainty into ERAs when extrapolated to other classes of chemistry. In this study, atrazine dermal absorption experiments were conducted with mallard, northern bobwhite, and rat skin. These data were used to derive an avian-mammal dermal route equivalency factor for atrazine and introduce an alternative approach for estimating dermal LD50 values and predicting exposure via the TIM dermal pathway. Compared to the default TIM method, use of measured rather than modeled data yielded TIM output with lower mean total dose, lower dermal fraction of total dose, greater oral fraction of total dose, and reduced model predicted mortality for atrazine. In addition, the new approach was compared with other methods for estimating avian dermal LD50 values, such as those proposed for use with mammalian data and physico-chemical properties. Ultimately, these comparisons demonstrated that the use of dermal route equivalency factor derived from empirical data provided estimates of avian dermal LD50 values that were markedly higher than those from model-based estimates. Consequently, use of dermal route equivalency factors based on atrazine-specific empirically derived data reduced predicted risk relative to those using default TIM model assumptions.

**RP033 Bird Survey Data Indicates No Negative Impact of Neonicotinoid Use**

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Neonicotinoids are currently one of the most widely used insecticide clasess. Although the risks to birds were evaluated and found to be acceptable by North American regulatory authorities when these products were first registered in the mid 1990's and early 2000's, it has recently been hypothesized that neonicotinoids may be having unforeseen adverse effects on avian populations, in particular on seed-eating and aerial insectivorous species. We assessed data from the North American Breeding Bird Survey (BBS) and the Iowa Breeding Bird Atlas (IBBA) to determine if there is evidence of direct (lethal or sublethal effects) or indirect (food chain disruption) impacts on these species. These data sets provide real-world observations of status and trends for avian species in landscapes where neonicotinoid use was formerly non-existent, but is now extensive. To determine if spatial or temporal population trends for aerial insectivore or seed-eating birds have been impacted, hierarchical models were fit to BBS data. In regions of high neonicotinoid use (*e.g.*, Prairie Pothole and Midwest Corn Belt), population trajectory for these species generally increased after the introduction of neonicotinoids. Analysis of data from individual BBS routes revealed no association between bird count data and percent of land area in crops receiving neonicotinoid treatment. Iowa has a high percentage of land area in crops receiving neonicotinoid applications. The IBBA provides data on the presence of bird species in "blocks" of land measuring 3 miles by 3 miles each that are systematically located across the state. Comparison of IBBA data for the years 1985-1990, before neonicotinoids were used, and 2008-2012, after neonicotinoids became widely used, revealed an increase in the % of blocks occupied after neonicotinoid use for 10 out of 11 species of aerial insectivorous and seed-eating species. The results of these analyses consistently found no evidence to support the hypothesis that widespread adoption of neonicotinoid insecticides has resulted in negative impacts on bird populations. If anything, bird population trajectories have increased since neonicotinoids were introduced.

## Assessing Contaminant Effects in Ecosystems with Multiple Stressors with a Focus on the California Bay-Delta

**RP034 Sediment Characteristics and Fluridone Concentrations: Building Upon Methods to Enhance Delta Smelt Habitat**

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The spread of the invasive aquatic plant, *Egeria densa*, in the San Francisco Bay-Delta has impaired water quality and habitat for the endangered Delta smelt, *Hypomesus transpacificus*. As part of the Delta Smelt Resiliency Strategy, the California Department of Water Resources partnered with California Department of Parks and Recreation, Division of Boating and Waterways (DBW) to determine if herbicide application could help improve degraded habitats of Delta Smelt. Since 2001 DBW has applied herbicides, including fluridone, to reduce the density and spread of *E. densa* and other aquatic invasive plants. Existing monitoring measures fluridone concentrations in water, but the primary route through which *E. densa* uptake fluridone is through the roots. We predict sediment fluridone concentrations will be greater than water concentrations and that sediment characteristics will affect flurdione concentrations. From October 2017 through June 2018, we measured fluridone concentrations and sediment characteristics at three sites: two treated sites (Decker Island and Little Hastings Tract) and one non-treated (French Island) in the northern region of the Bay-Delta. Fluridone concentrations were significantly greater in the sediment than in the water at both treated sites. Fluridone concentrations ranged from non-detect to 93 µg/L in sediment, compared to non-detect to 6.60 µg/L in water. Decker Island, located in the Sacramento River, had significantly lower organic content than French Island and Little Hastings Tract. Over fifty percent of the sediment composition ranged in size from 5 and 7.5µm , with less than 15% of the sediment being less than 1 µm. Results from this study will help inform aquatic invasive plant control programs using fluridone enhance the effectiveness of *Egeria* management in the San Francisco Bay-Delta.

**RP035 From algal toxins to environmental DNA: Passive samplers as a tool to help with multiple management objectives**

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Climate changes are expected to affect water-quality, ecosystem health, and species viability within the San Francisco Bay-Delta (Delta). As these changes occur, it will be necessary to streamline data collection protocols so that sampling tools can simultaneously address multiple management objectives while saving time and money. One currently used tool that shows promise for meeting multiple management objectives are Solid Phase Adsorption Tracking (SPATT) samplers. SPATTs were developed as an economical tool to passively monitor algal toxins that may be missed by discrete grab sampling. SPATT's have proven useful in the Delta and will continue to be used to monitor future algal toxins. We found SPATTs can also be used to monitor for Environmental DNA (eDNA). The eDNA method is an efficient, non-invasive, and relatively rapid process that can determine species presence and organism occupancy. Typically, eDNA monitoring involves collecting a discrete grab sample. However, traditional sampling methods may limit the spatial and temporal scale of eDNA monitoring. In a pilot study, we used SPATT and grab samples to determine if imperiled freshwater mussels (Unionidae) were present in the Delta. We deployed six SPATTs at two Delta locations. *Anodonta Californiensis* were detected in all six SPATT samples and 33 grab samples in the San Joaquin River near Mossdale. No mussel eDNA was detected in any SPATT samples at the I street bridge, but *A. Californiensis* was detected in 2 of 11 grab samples. This is the first time we are aware that SPATT samplers have been used to monitor for eDNA. Although we successfully used SPATTs, additional eDNA investigations and modification of the method may further improve upon our results. We believe this is a promising tool to meet future management objectives by cost effectively detecting cryptic species such as Delta Smelt, while simultaneously monitoring for algal toxins.

**RP036 Networks of Disturbance of Midwestern United States Streams**

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The National Water Quality Assessment Project (US Geological Survey) surveys streams in different eco-regions to better understand how land use practices affect water quality and ecological integrity. In the summer of 2013, 100 streams in the Midwestern US were sampled. Several modeling approaches (e.g., CART, BRT, and Quantile Regression) were previously employed to filter over 2000 indicators (e.g., GIS variables, water and sediment chemistry, and habitat) of ecological condition. Through this empirical process we identified fifteen indicators that were important to stream condition. Employing watershed theory, we developed a structural equation model (SEM) of how these indicators were inter-related. Then using SEM, we fit this “disturbance network” to multimetrics of algae, invertebrate, and fish community integrity removing any indicators otherwise not related to the ecological responses. Six of the fifteen indicators—agricultural and urban land uses, sand content of watershed soils, riparian forest area, soil eroded into the stream, and relative bed stability—were important to all three-community metrics. The algae (riparian cover, temperature, phosphous, total triazine) and invertebrate (nitrogen, ammonia, pyrethroid degradates in water, bifenthrin in sediment) models included unique variables not included in the fish model. Although all three landscape indicators were important predictors of all the ecological communities, agricultural (algae), urban (invertebrates), sand content of watershed soils (fish) were most strongly related. Ecological integrity of midwestern streams is affected by both agricultural and, surprisingly, urban land uses. Synthetic chemicals related to crops (pesticides and nutrients) and residential uses (pyrethroids) are more strongly related with ecological integrity than natural factors (riparian forest, watershed soil character), however, these natural factors are important to the ecological integrity of streams despite the prevalence of intense agricultural land use in the midwestern US.

## Environmental Mercury Exposure: Mechanisms Associated with Its Immediate and Generational Effects

### RP038 Mercury concentrations in subsistence foods from Lake Atitlán, Guatemala

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Lake Atitlán, the deepest lake in Central America, is a crucial source of subsistence foods and supports productive agricultural systems. Anthropogenic contamination has accelerated in recent decades due to untreated wastewater discharge and fertilizer runoff that enters the lake. Elevated concentrations of heavy metals, including mercury (Hg), may pose a health risk to local wildlife and the 250,000 inhabitants of the Lake Atitlán watershed. Hg is a neurotoxic metal that disrupts normal reproductive and neurological function in humans. Hg has the potential to bioaccumulate and biomagnify within the food web, posing additional risks to the Lake Atitlán community. This study aims to estimate current Hg levels in important subsistence foods (fish, snails, and crabs). Samples were collected from the Santiago market that sells Lake Atitlán catch for human consumption. Hg levels in muscle tissue from each organism are analyzed using the PerkinElmer FIMS 100 Flow Injector Hg system. Tissue samples are freeze dried, digested using hydrochloric acid, filtered, and analyzed for Hg content. High detectable Hg levels in these organisms may exceed the EPA guidelines for safe Hg consumption. These results will indicate if heavy metal contamination in the watershed has resulted in elevated concentrations in local wildlife and food sources that support a diverse group of Mayan cultures.

### RP039 Role of natural organic matter in regulating the toxicity of MeHg in embryonic Zebrafish

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Dissolved organic matter (DOM) occurs ubiquitously in aquatic environments and plays important roles in regulating the solubility and chemical speciation of metals and thus their fate, transport, bioavailability and toxicity. Over the past decades, the toxicity, bioaccumulation and biomagnification of mercury (Hg), including inorganic Hg and methylmercury (MeHg), have been extensively studied in many aquatic organisms. However, interactions between natural DOM and metals, and changes in metals chemical speciation and toxicity, remain poorly understood. In this study, we have examined the mitigation effects of natural and model DOMs on the toxicity of Me-Hg in embryonic zebrafish. Five model DOM with different functionalities, including thiosalicylic acid (TA containing aromatic thiol), dextran (DX, a disaccharide), alginic acid sodium salt (AAS, polysaccharide with –COOH), L-glutathion (GSH, peptide with thiol), and humic acid (HA), and three natural organic matter (NOM) samples, including Suwanne River NOM, Yukon River NOM and Mississippi River NOM, were used to elucidate the role of DOM quantity and quality in mitigating Me-Hg toxicity. The embryonic Zebrafish (< 4 hpf) were exposed in media with DOM (at 0, 1, 3, 10, 30 and 100 mg-C/L, respectively) and MeHg (at 0 and 300 nmol-Hg/L) for 24 h. After the initial exposure, the embryos were then exposed in medias with DOM without MeHg. Hatching, mortality and deformation at 24, 48, 72 and 96 h were recorded. The results showed that the mortality of embryos exposed in the MeHg was up to 30% at 24 h, and deformations at 72 and 96 h were almost 100%. Main deformations were brain and tail, followed with axis, face, eye and cardiac edema. With increasing concentrations of DOM (except for DX), the extent of deformations on the axis and tail was mitigated. The deformation of larvae exposed to AAS decreased with increasing DOC concentration (from 98% to 27% at 72 h and from 90% to 29% at 96 h). In addition, the GSH and TA at the concentration of 10 ppm and 1 ppm-DOC could effectively alleviate the toxicity of MeHg at 72 h, 3 ppm for GSH and 1 ppm for TA at 96 h. Three NOM also mitigated the toxicity of MeHg on the embryonic Zebrafish. Therefore, our results demonstrated that natural DOMs with different functional groups could effectively mitigate, to various extents, the toxicity of MeHg in the zebrafish, with thiosalicylic acid containing aromatic thiols being the most effective to alleviate the deformation of fish.

### RP040 The use of lichen as a bioindicator of atmospheric monomethylmercury deposition in coastal California

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Monomethylmercury (MMHg) is a potent neurotoxin that bioaccumulates in food webs and was recently discovered to be present in marine fog and stratus clouds on the coast of California due to the oceanic evasion of a volatile precursor compound called dimethylmercury. The goal of this project was to determine if a commonly-found species of lichen (Ramalina menziesii) could serve as a bioindicator of atmospheric MMHg and total Hg (THg) deposition in coastal California. For this project, 29 Ramalina menziesii samples from 14 different state and county parks located 0-60 km inland from the ocean, were collected, freeze-dried and homogenized, then analyzed for MMHg and THg concentrations. The THg concentrations ranged from 49.5 to 1353.6 ng g-1, with an average of 227.4 ± 251 ng g-1. The MMHg concentrations ranged from 3.5 ng g-1 to 65.2 ng g-1, with an average of 19.6 ± 15 ng g-1. Average MMHg concentration for lichen from each site increased significantly with the fog and low cloud cover (hours/day) at each site (ANOVA, p= 0.0004), as determined from literature data. The sites located 0-10 km to the ocean had a mean MMHg concentration that was 3.7 times higher than the sites located 30-60 km from the ocean. These data suggest that lichen is a good indicator of airborne mercury concentrations and that some species, like Ramalina, which are eaten by deer and other animals, could expose higher trophic animals like mountain lions to unsafe levels of Hg in their diets.

**RP041 Mercury Concentrations and Seafood Mislabeling in Sashimi Purchased in Central Texas**

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Sushi consumption in the U.S. has grown significantly and while providing many important health benefits, sushi including sashimi, can be a significant source of mercury (Hg) to humans. Seafood mislabeling, which can potentially expose consumers to elevated levels of Hg, makes assessing the risks and rewards of seafood consumption even more complex. The objective of this study was to 1) determine the concentration of Hg in sashimi [tuna, albacore, white/super white tuna, salmon, red snapper, yellowtail, Japanese snapper, octopus, squid, and roe (salmon and sea urchin)] purchased from restaurants in Central Texas using a direct mercury analyzer, and 2) genetically identify the species used in sashimi using DNA barcoding. Mean Hg concentrations, based on restaurant menu items, was highest in white/super white tuna, Japanese snapper, and tuna (0.968, 0.595, and 0.539 µg/g wet wt respectively), and lowest in squid, salmon, sea urchin roe and salmon roe (0.022, 0.022, 0.005, and 0.002 µg/g wet wt, respectively). 46% and 14% of white/super white tuna and tuna samples, respectively, exceeded the 1 µg/g wet wt Food and Drug Administration (FDA) action level. The variability in Hg concentration for a given type of sashimi could be due to differences in body length/age, harvesting location, trophic level and mislabeling, which makes assessing the potential risk of sashimi consumption difficult. Of the 115 samples which could be genetically identified, 48% were mislabeled; 100% of white/super white tuna, red snapper, Japanese snapper, yellowtail, and squid were mislabeled, whereas tuna, salmon, octopus and roe were all correctly labeled. Mislabeling resulted in sashimi samples having Hg concentrations that did not correspond to expected values based on menu listings. Red snapper for example was identified as either Mozambique tilapia or red seabream, which resulted in samples having a lower Hg concentration than what was expected. In comparison, white/super white tuna was actually escolar which resulted in a higher than expected Hg concentration. High instances of seafood mislabeling across a variety of sashimi types, such as those reported in the present study, are concerning and warrant further study.

**RP042 Selenium Availability in Freshwater Fish is Inversely Related to Methylmercury Bioaccumulation and Toxicity**

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Based on the biochemical mechanisms of mercury (Hg) toxicity, concomitant assessments of selenium (Se) intakes are required for reliable assessments of risks potentially associated with Hg exposures. This consideration is especially true in regions that are Se-poor or exposed to high Hg inputs. In particular, subsistence consumers of high methyl-Hg ($CH_4Hg^+$), low-Se freshwater fish may be at greater risk than would have been assumed based on earlier assumptions that did not consider Hg's biochemical mechanisms of toxicity. Because $CH_4Hg^+$ is uniquely able to: 1) diminish maternal Se redistribution across the placental barrier, 2) decrease Se-transport across the blood-brain barrier, and 3) irreversibly inhibit selenoenzyme activities which are required to prevent and reverse oxidative damage in brain tissues, Se status is a pivotal consideration in $CH_4Hg^+$ risk assessments. The Hg and Se content data of ~5,000 freshwater fish from North American watersheds were compiled for calculation of their Health Benefit Values (HBVs). There was a strong inverse relationship between environmental Se availability and $CH_4Hg^+$ contents of the fish, especially among aquatic apex predators. Due to the high binding affinities between Hg and Se, the same chemical reactions responsible for the irreversible inhibition of Se-dependent enzymes, formation of insoluble HgSe, and the physiological consequences of high $CH_4Hg^+$ exposures were predicted to result in an inverse relationship between Hg and environmental Se availability. In the presence of adequate Se, Hg that is retired as HgSe cannot bioaccumulate in aquatic food webs. However, in the absence of adequate Se, there is no Hg retirement, and increased $CH_4Hg^+$ bioaccumulation should occur. This study found the highest fish Hg concentrations were observed in watersheds with the poorest Se availability. Although increased exposure from eating such fish will increase risk, the fact that Se-poor consumers will be more vulnerable to the effects of high $CH_4Hg^+$ exposures accentuate the risks of adverse effects. Thus, increased $CH_4Hg^+$ exposures in areas where environmental Se is poorly available may result in an adverse synergy with the potential to cause far greater impairments to fetal neurodevelopment than those predicted by earlier $CH_4Hg^+$ risk assessment criteria. Increased monitoring is required to identify regions that are likely to have subsistence populations at risk.

## Plants in Environmental Risk Assessment: Assessing and Predicting the Effects of Chemicals on Plant Communities

**RP043 Determining the size effect that can be estimated from a NTTP Study**

*J.W. Green, DuPont / Data Science and Informatics*

Regulatory requirements in the EU and US on the size effect to be estimated for responses (growth and percent emergence) from guideline non-target terrestrial plant (NTTP) studies are under review. Such studies are conducted primarily to determine whether crop protection chemicals meant to protect a specific crop might cause harm to other plant species that are unintentionally exposed, such as through spray drift. Crop Life America investigated a large database of such studies from testing facilities across the world with the aim of determining what regulatory requirements are justifiable and consistent with data routinely collected in such studies. One issue investigated was for what values of p is it reasonable to require the estimation of a p% effects rate (ERp) for growth and shoot height measurements for regulatory risk assessment. To address the question, seven regression models were attempted to be fit to both responses from vegetative vigor and seedling emergence studies in the database and the quality of the models were assessed using well-defined objective criteria (Green *et al.* 2018). The best model was selected for each response and ECx estimates were assessed for x=5 to 50 from that model. This allowed an assessment of the size effect that can reasonably be expected for these responses and study types. Models investigated are those recommended in OECD guidance (OECD 2014) on statistical analysis of ecotoxicity studies and have a long history of use in numerous NTTP studies. These are the Bruce-Versteeg, Brain-Cousens hormetic, 3-parameter log-logistic, and a suite of 2-4 parameter exponential models. There were 89 studies, and approximately 10 plant species tested per study and 2 responses per plant, so well over 10,000 regressions evaluated. The results of this study provide a robust guide to the size effect can be estimated under a variety of conditions regarding dose-response shape, maximum observed effect, and experimental design. In addition, model selection and estimate assessment criteria went through a rigorous test and specific recommendations are provided.

**RP044 Hydroponic uptake and distribution of PPCPs in *Typha lafitolia* and *Zea mays*: A comparative study in two monocotyledon model plants**

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Pharmaceuticals and personal care products (PPCPs), originating from wastewater treatment plants, have been detected in reclaimed waters and aquatic ecosystems potentially resulting in the accumulation of PPCPs in wetland plants and terrestrial crops. The objective of this study is to investigate the root uptake, translocation and distribution of a mixture of PPCPs and the herbicide atrazine, in two monocotyledon plants, one wetland plant *Typha latifolia* and one terrestrial plant *Zea mays*. The target PPCPs included antibiotics (sulfamethoxazole (SMZ) and triclosan

(TRI)), an anticonvulsant (carbamazepine (CBZ)), an antidepressant (fluoxetine (FLX)), and an antihyperlipidemic (gemfibrozil (GBZ)). Atrazine (ATZ) was also selected as a herbicide commonly used on corn. Twelve size uniform plants of each specie were selected for the hydroponic exposure assay. Six plants were used as untreated controls and six were treated. A single dose was added to the hydroponic containers to yield an initial concentration of 20 µg/L for each compound. Transpiration was measured by water lost in the root-zone vessels relative to a non-planted control. The root zone solution was replenished weekly with a nutrient solution containing 20 µg/L of the target compounds. For *T.latifolia,* root, rhizome, sprout, stem and leaf (base, middle and tip) tissues were collected and analysed while root, stem, base and top leaves and bud flowers were examined for *Z.mays*. Sample extracts were analysed using liquid chromatography coupled to a tandem mass spectrometer. All target compounds were detected in the roots of both plants. Above ground tissue concentrations were normalized to the amount of water transpired. For *T. latifolia,* only PPCPs and ATZ were found in the stems while the distribution of target compounds in the leaves *had the following* pattern: CBZ was accumulated in the mainly tip FLX mainly in the base and ATZ and TRI more uniformly in the all leaves. In corn leaves CBZ, FLX and ATZ were mainly accumulated in the base leaves and the transpiration normalized concentrations were lower than for *T.latifolia*. The rhizoma and sprouts of *T.latifolia* also contained measurable concentrations of the target compounds. No target compounds were detected in corn bud flowers. The results show the importance of examining the distribution of contaminants within the various plant tissues before conducting risk or biomonitoring assessments.

### RP045 MDD% as a guide to the size effect that can be estimated from regression

*J.W. Green, DuPont / Data Science and Informatics*

The concept of minimum detectible percent difference, MDD%, was explored for its relevance to the size effect that could be expected to estimate from regression. MDD% was developed for hypothesis testing and is routinely used in meso/microcosm studies, where few treatment groups are typically available, making regression analysis suboptimal at best. It is relatively straightforward to extend the MDD% concept to a simple linear regression model in studies with an ample number of treatment groups. In Staveley *et al.* (2018), it was shown that with suitable modification it can be extended to nonlinear models. The results of this study are used to explore the utility and limitations of MDD% in predicting the size effect that can be estimated reliably in a NTTP study. At the design stage, there is often a historical control database that indicates the typical variability on a per-species basis for each response of interest. To the extend MDD% is a reliable guide to what can be estimated, this information can serve at least two purposes. First, it informs the risk assessor what can be obtained from such studies. This will help in distinguishing between a real lack of effect and poor experimental design or analysis. Second, it may help in the selection of plants species to include in a study. If the test chemical has similar modes of action on two plant species but notably different variability leading to different size effects that might be estimable, then the species with lower variability might be preferred if it is acceptable on regulatory grounds to choose either species. A large database of non-target terrestrial plant (NTTP) studies collected by a team sponsored by Crop Life America was used to determine the extent to which the modified MDD% provided a reliable guide to the size effect that can be estimated reliably from a typical non-linear regression model fit to the data. References Integrated Environmental Assessment and Management Staveley JP, Green JW, Nusz J, Edwards D, Henry K, Kern M, Deines AM, Brain R, Glenn B, Ehresman N, Kung T, Ralston-Hooper K, Kee F, McMaster S 2018. Variability in nontarget terrestrial plant studies should inform endpoint selection. Integrated Environmental Assessment and Management. https://doi.org/10.1002/ieam.4055.

### RP046 Phytoplankton and cyanobacteria growth inhibition by glyphosate, imazamox and fluridone herbicides

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Phytoplankton and cyanobacteria are key primary producers that form the base of the aquatic food web. In the Sacramento-San Joaquin Delta, primary productivity is low, limiting food availability for higher trophic levels. Blooms of toxin-producing cyanobacteria also occur in the summer and fall. Additionally, herbicides are employed yearly in the Delta to control invasive aquatic plants that block waterways and degrade habitat quality. Whether or not beneficial or harmful algae growth will also be inhibited by these herbicides is of interest due to the potential impact through trophic cascade to fishery production. For this study, we used 96-well plate growth tests to determine if algae growth is inhibited by the herbicides: glyphosate, imazamox and fluridone, which are currently used to control invasive aquatic plants in the Sacramento-San Joaquin Delta. For the growth inhibition tests, we used three species found in and/or isolated from the Delta: *Thalassiosira pseudonana* (diatom), *Microcystis aeruginosa* (cyanobacteria) and *Chlamydomonas sp.* (green algae). Growth was tracked daily by measuring optical density in the 96-well plates over five days. The extent of algae growth inhibition varied depending on the test species and the herbicide tested. We found that glyphosate and imazamox inhibited growth of all species at concentrations higher than what would be found in the environment, inhibiting growth between 7,000 ppb to 70,000 ppb, and between 20,000 ppb to 200,000 ppb respectively. However, fluridone inhibited growth of all algal species in concentrations at a lower environmentally relevant range of 10 to 300 ppb. *M. aeruginosa* and *T. pseudonana* were more sensitive than *Chlamydomonas* to all of the herbicides. Based on the varying sensitivities among algal species and herbicides, additional growth inhibition tests with a variety of native algae species can help managers identify contaminants and contaminant levels that will negatively impact beneficial or harmful species present in the Delta. Of the three herbicides tested, fluridone was the only chemical that inhibited algal growth at concentrations that could potentially be applied in the environment. Thus, fluridone treatments may decrease pelagic primary productivity in the Delta, although further experiments with field water are needed.

### RP047 The effect of buffering systems on pH-drift and Myriophyllum spicatum growth in OECD TG 239 tests

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The aim of this research was to improve the already existing OECD TG 239 *Myriophyllum spicatum* water-sediment toxicity test guideline by exploring methods to avoid or minimize pH-drifts. These pH-drifts normally occur when macrophytes are grown submerged in toxicity tests. On the one hand, pH and inorganic carbon resources are strongly interdependent and influence the growth of macrophytes. On the other hand, tested herbicides may be hydrolized because of increasing pH. In the experiment, pH drift and growth of *M. spicatum* were compared among control treatments, $CO_2$-bubbled systems, air-bubbled systems, and two treatments containing two different chemical buffers, i.e. MOPS (3-(N-morpholino) ethanesulfonic acid), and MES (2-(4-morpholino) ethanesulfonic acid). The test was performed twice. The first experiment showed a number of aberrations and low growth of the *Myriophyllum* shoots. In order to improve the set-up of the first experiment, a second experiment was performed with spring shoots and slight modifications. The $CO_2$-bubbling system resulted in a pH of around 6.0. Contrary to the first experiment, the air-bubbling system did not have an effect on pH. The MOPS and MES buffers managed to keep the pH around constant values of 7.5 and 6.9, respectively. However, the growth in these systems tended to be lower than in the control and the bubbled treatments. The growth in controls showed a RGR of dry weight of 0.04 ± 0.03 $g.g^{-1}.d^{-1}$ for the total shoots while this value was 0.081 $g.g^{-1}.d^{-1}$ for the $CO_2$-bubbling treatments and 0.066 $g.g^{-1}.d^{-1}$ for the air-bubbling systems. These results are comparable to those found in in literature. The increased growth in the bubbling systems tended to show that an input of $CO_2$ in the medium *via* a $CO_2$-tank or the ambient air allowed $CO_2$ assimilation which is a lower cost for submerged macrophytes compared to the use of $HCO_3^-$.The air-bubbling did not manage to maintain the pH at a constant range. It is recommended not to use chemical buffers because of a deleterious effect on *M. spicatum* growth. Further experiments are needed. For example, using a mixture of air and $CO_2$ could be a way forward to achieve a constant but higher pH. At the end, maintaining a pH constant over the duration of the experiment could ensure a proper growth of the macrophyte and avoid a fast degradation of the herbicide, leading to an unbiased exposure of the macrophytes to the herbicide.

**RP048 Acetaminophen Detoxification in Plants via Induction of Glutathione S-transferases**

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Many pharmaceutical and personal care products (PPCPs) enter agroecosystems during reuse of treated wastewater and biosolids. Research on the impact of pharmaceuticals in the environment has mainly focused on determining persistence, transport and concentrations in the soil; little work has been conducted to elucidate the mechanisms of pharmaceutical uptake, accumulation and detoxification by plants. Here, acetaminophen, one of the most-used pharmaceuticals, was used to explore roles of glutathione (GSH) conjugation in its biotransformation in crop plants. Acetaminophen was taken up by plants, and conjugated quickly with GSH. After exposure to 5 mg $L^{-1}$ acetaminophen for 144 h, GSH-acetaminophen conjugates were 15.2±1.3 nmol $g^{-1}$ and 1.2±0.1 nmol $g^{-1}$ in cucumber roots and leaves, respectively. Glutathione-acetaminophen was also observed in common bean, alfalfa, tomato, and wheat. Inhibition of cytochrome P450 decreased GSH conjugation. Moreover, the GSH conjugate was found to further convert to cysteine and N-acetylcysteine conjugates. Glutathione S-transferase activity was significantly elevated after exposure to acetaminophen, while levels of GSH decreased by 55.4% in roots after 48 h, followed by a gradual recovery thereafter. Enzymes involved in GSH synthesis, regeneration and transport were consistently induced to maintain the GSH homeostasis. Therefore, GST-mediated conjugation likely played a crucial role in minimizing phytotoxicity of acetaminophen and other PPCPs in plants.

## Environmental Assessments for Human and Veterinary Pharmaceuticals – Evolving Regulations

**RP049 Effect of Differing Regulatory Guidance on Acceptable Levels of Active Pharmaceutical Ingredients in Industrial Wastewater Discharges**

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Wastewater discharge permits may not specifically regulate chemicals that are unique to the pharmaceutical and animal healthcare industries, such as active pharmaceutical ingredients (APIs). However, it is recognized that uncontrolled release of these chemicals has the potential to adversely affect the environment in areas downstream of the discharge. This issue can be addressed by determining acceptable concentrations of APIs and applying them to the downstream location where an exposure could potentially occur. However, a globally harmonized approach for the derivation and application of such acceptable concentrations does not exist, which can lead to substantial differences in the conclusions of risk assessments. To explore this issue, acceptable concentrations to which populations of aquatic organisms, wildlife, and humans could be exposed were calculated for a variety of APIs spanning several pharmaceutical classes using two approaches: USEPA's Great Lakes Initiative (40 CFR 132) and the European Union's Water Frame Work Directive (2000/60/EC) methods for deriving water quality standards. Acceptable concentrations were calculated using risk-based methods for several receptor-exposure scenario combinations, including acute and chronic exposure for aquatic organisms, chronic exposure for wildlife, and chronic exposure for humans associated with drinking water and recreational exposure scenarios. A sensitivity analysis was performed to evaluate the influence of the input variables and uncertainty in the underlying datasets on the calculated acceptable concentrations. Additionally, the effect of using US and EU-based approaches for evaluating the dilution capacity of receiving waters on the calculation of acceptable daily water discharges was compared. Recommendations are provided to help reduce uncertainty in the derivation of acceptable concentrations and wastewater discharge rates for APIs.

**RP050 Uptake and physiological responses to a model calcium channel blocker in fathead minnow across a dissolved oxygen gradient**

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Urbanized watersheds routinely represent worst case scenarios for fish exposure to multiple stressors, including contaminants of emerging concern (e.g., pharmaceuticals) and depressed dissolved oxygen (DO). Field observations from our laboratory identified concentrations of diltiazem, a calcium channel blocker used to treat high blood pressure, in fish plasma exceeding human therapeutic levels within multiple urban estuaries of the Gulf of Mexico. Additionally, these estuaries are commonly listed on the Texas, USA 303(d) list for non-attainment of DO water quality criteria. Therefore, we examined physiological responses to diltiazem across typical DO water quality criteria (WQC) values and developmental stages in the fathead minnow. Our results indicate DO significantly increases larval diltiazem toxicity following acute (48 h) exposures. DO levels (3.0 mg DO/L) consistent with current WQC values significantly doubled the uptake of diltiazem in adult fish relative to normoxic conditions (8 mg DO/L). Increased internal diltiazem concentrations were associated with significant increases in fish ventilation rate at low DO levels. Concomitant physiological responses revealed DO significantly decreased bursting swim performance ($U_{burst}$) by 12-31 % across diltiazem treatments. Such physiological effects paired with internal diltiazem tissue concentrations inform comparative physiology, pharmacology, and toxicology approaches to identify adverse outcomes, and further demonstrate the utility of employing model pharmaceuticals for basic and applied research.

## California Oil Spills: Impacts on Habitat and Wildlife

**RP051 Toxicity of High Energy Water Accommodated Fractions of Refugio Oil to Sand Crabs, Blue Mussels, and Inland Silversides**

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On May 19, 2015, the Refugio Beach Oil Spill occurred along the Gaviota Coast of California. As part of the evaluation of the effects of the spill on wildlife, aquatic toxicity studies were performed with multiple test species. As sand crabs, *Emerita analoga,*reside throughout the beaches impacted by the spill, sand crabs from an unimpacted population from Sonoma Creek Beach were collected to evaluate oil exposure effects on resident organisms via a 6-day test for which survival and growth (weight, length, incremental growth) were measured. In addition, the blue mussel, *Mytilus galloprovincialis*, larval development test and *Menidia beryllina* survival and growth test were conducted following standard EPA test protocols. All three organisms were exposed to high-energy water accommodated fractions (HEWAFs) prepared daily using filtered seawater and Refugio source oil following protocols established by the National Oceanic and Atmospheric Administration; a blended control was similarly prepared. Daily HEWAF solutions and filtered seawater were used to prepare nominal dilution series of 0.5, 1, 5, 10, 50, 100, and 500 µg/L. Significant reductions were observed in the *M. galloprovincialis*embryo development,*E. analoga* survival and growth (i.e., biomass, length, and incremental growth), and *M. beryllina*survival and mean dry weight test endpoints. The general order of sensitivity of the test species to the Refugio WAFs was *E. analogo*> *M. beryllina*>> *M. galloprovincialis*.

**RP052 Western Snowy Plover Reproductive Effects Associated with the Refugio Beach Oil Spill**

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During the May 2015 Refugio Beach oil spill, federally threatened western snowy plovers (*Charadrius nivosus nivosus*) were feeding with their recently hatched chicks on southern California beaches. They primarily forage on invertebrates in the wrack and wave-washed swash zone and were directly threatened by both the oil and the actions of the clean-up crews. During the spill, the extent and location of oiling observed on western snowy plovers at Coal Oil Point Reserve, in Santa Barbara, were documented. In addition, annual monitoring of reproductive success was conducted (i.e., number of nests, number of eggs laid, number chicks fledged, etc.), contributing to data collected on this population since 2001. Although no direct mortality of western snowy plovers was observed during the spill, a spike in the number of infertile eggs was observed in the year following the spill. As part of the Natural Resource Damage Assessment, estimated oil ingestion rates were compared to exposures associated with reproductive harm in other avian oil toxicity studies. A plausible connection between oil ingestion via preening and feeding and the increase in egg infertility in Western Snowy Plovers at Coal Oil Point Reserve was demonstrated.

**RP053 Marine mammal strandings following the 2015 Refugio Beach Oil Spill in Santa Barbara County, CA**

*S. Chivers, S. Wilkin, NOAA Fisheries; L. Henkel, California Department of Fish and Wildlife; L. Sullivan, NOAA / ORR /ARD*

An estimated 190,000 liters of crude oil entered the surf zone near Refugio State Beach in Santa Barbara County from an on-shore oil pipeline spill on May 15, 2015. Once in the ocean, the oil fouled local beaches and nearshore waters. The wildlife response team responded to reports of live and dead oiled marine mammals following the spill and recovered 162 marine mammals from Santa Barbara and Ventura County shorelines. Among the mammals recovered, there were 63 live pinnipeds, and 99 dead pinnipeds and dolphins. Of the 63 live pinnipeds captured and transported to rehabilitation centers for care, 23 were released. The primary species recovered was the California sea lion. The assessment of injuries to marine mammals resulting from the oil spill included a review of all wildlife intake records for recovered mammals and a comparison of them to historic records for Santa Barbara and Ventura county beaches. A summary of marine mammal stranding response activities and of the methods used to assess injury to mammals from the oil spill will be presented.

## Chemical Prioritization Using 21st Century Science: Considerations for New Approach Methodologies

**RP054 Application of Mass Balance Models to Understand the Behaviour of Organic Chemicals in In Vitro Toxicity Tests**

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Toxicity testing in the 21st century is expected to rely increasingly on in vitro assays, which now cover a wide range of endpoints including cytotoxicity, receptor binding, protein interactions and DNA binding. In most cases, dose-response relationships from in vitro toxicity tests are reported using nominal concentrations in the test medium despite the known challenges such data introduce for comparing results across different test conditions and between different chemicals. With respect to existing data reported on a nominal basis (e.g., ToxCAST™), one option to further probe reported results is to apply mass balance models to simulate the distribution of the chemical in the test system. Indeed, equilibrium partitioning (EQP) and toxicokinetic (TK) models have already been published in the literature for this purpose. The main objective of this study was to develop and apply EQP and TK models under both generic (hypothetical) and actual in vitro toxicity test conditions to better understand the potential behaviour of organic chemicals in these systems. For relatively persistent chemicals (or in cells/tissue with limited metabolic competency), the simulated mass distribution using the TK model is similar to the equilibrium partitioning model output for test durations greater than 12 h. Based on these results, we then applied the EQP model to a specific ToxCast assay (ACEA_T47D_80hr_negative assay; cytotoxicity) to illustrate the value of this modelling approach. For approximately 300 neutral organic chemicals with “Hits” (i.e., reported AC50s) included in these simulations, approximately 2/3rds had predicted membrane concentrations (at the AC50) in the range consistent with baseline toxicity (membrane dysfunction/narcosis). Chemicals with predicted membrane concentrations well below the baseline toxicity range may act via a specific mode of action and could therefore be prioritized for further investigation. The EQP model also provided insights into the approximately 900 chemicals with “Misses” (no response) in the same assay

**RP055 Comparing Mode of Action (MOA) classification using body residues, membrane concentrations and chemical activity for chemical prioritization**

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In 2016, Environment and Climate Change Canada (ECCC) released the first version of an IATA-like Ecological Risk Classification system (ERC1) for prioritizing the organic substances on the Canadian Domestic Substances List (DSL) remaining to be assessed before 2020. One of the key considerations for assessing toxicity in ERC1 is a Mode of Action (MOA) classification which aims to distinguish between baseline toxicants (“narcotics”) and specifically-acting toxicants in a given test organism/receptor. As part of the MOA classification for aquatic organisms (fish), ERC1 used an estimate of the Critical Body Residue (CBR) corresponding to an empirical or QSAR-based lethal concentration (LC50). Two alternative/complementary metrics for MOA classification that have been advanced recently are i) Critical Membrane Concentration (CMC) and ii) (lethal) chemical activity (La50). At the screening level, the CBR, CMC and La50 approaches rely on different physical-chemical properties to “translate” the LC50, namely octanol-water partitioning ($K_{OW}$), membrane-water partitioning ($K_{MW}$) and water solubility respectively. The main objective of this study was to generate CBRs, CMCs and La50s for a set of 929 neutral organic chemicals with empirical LC50 data and compare the MOA classifications across the three metrics and to the MOA classification based on another approach applied in ERC1 (structural alerts). Complete agreement in the screening level MOA classification using CBR, CMC and La50 was observed for 719 of 929 chemicals (599 baseline toxicants, 120 specifically-acting). Complete agreement in the screening level MOA classification using CBR, CMC, La50 and structural alerts was observed for 585 of 929 chemicals (522 baseline toxicants, 63 specifically-acting). Explanations for the apparent discrepancies include uncertainties in property measurements/estimates, problematic toxicity data (e.g., LC50s greater than water solubility) and the domains of applicability for all approaches (including MOAs based on structural alerts). The findings of this exercise will be used to inform version 2.0 of the ERC, which is being developed as one approach to determine chemical priorities for post 2020 chemicals management in Canada.

## Soil Contaminants: Fate, Bioavailability, Environmental Toxicology in Ecological and Human Health Risk Assessment

### RP056 Can soil invertebrate traits help us understand interspecies differences in responses to petroleum hydrocarbon contaminated soils?

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Soil invertebrates display a range in interspecies sensitivities to petroleum hydrocarbon contaminated soils in terms of mortality, reproduction and avoidance. For instance, in our studies we found, mortality of two phylogenetically similar species, *Eisenia fetida* and *Enchytraeus crypticus*, exposed to petroleum hydrocarbon contaminated soils ranged several orders of magnitude, from 2,860 to over 186,000 mg/kg total petroleum hydrocarbons. With many contaminants, the avoidance response is typically as sensitive as reproduction, however, we found the predatory mite, *Hypoaspis aculeifer,* avoidance an order of magnitude greater than its reproduction. Trait based approached have been a successful tool in monitoring community changes following exposure to low concentrations of pesticides in German rivers. We qualitatively discuss how traits explain the interspecies variations of six soil invertebrates responses to petroleum hydrocarbon contaminated soils.

### RP057 Environmental risk analysis for crude oil soil pollution

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Environmental risk assessment (ERA) is predominantly a scientific activity and involves a critical review of available data for the purpose of identifying and possibly quantifying the risks associated with a potential threat. Risk management (RM) is performed to consider the need to impose measures to control or manage the risk. The working methodology presented in this paper was done based on several research studies for the environmental risk assessment for soil pollution with hidrocarbures from accidental crude oil spills. The qualitative and quantitative assessment of the environmental risk for industrial sites for drilling, gas oil separation it is complex and require several data. For these reasons an environmental risk calculation methodology for soil is presented which is structured in modules and steps. Environment risk assessment of pollution with hydrocarbons from crude oil comprises five interrelated modules: hazard identification, hazard assessment, risk estimation by the award of "grades" for the frequency and severity of consequences environmental risk assessment based on risk criteria (ALARP) and environmental risk management. The modules required data: technical data for the equipment in the upstream industrial activities, extraction and gas-oil separation, physico-chemical analysis for the soil contaminants, soil properties that may influence the severity and consequences of the default risk, charts, mathematical equations and matrix assessment of environmental risk intensity. In the methodology are established the steps needed to calculate the alert threshold and intervention and additional studies needed (geotechnical study, pedological and chemical study).

### RP058 Polycyclic Aromatic Hydrocarbon Accumulation in Soil throughout Oklahoma City, Oklahoma

*S. Hileman, J.B. Belden, Oklahoma State University / Integrative Biology*

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in the environment and have been found to be of concern with regards to human health. PAH contamination may correspond with atmospheric deposition, and in urban environments soils may contain elevated levels due to proximity to combustion sources. Sampling in the Oklahoma City Metropolitan Area has indicated that PAH accumulation (and especially carcinogenic PAHs or cPAH concentration) has been significant, and in many cases cPAH load has been measured above the USEPA's residential soil screening level of 110ppb. The Oklahoma City Metropolitan Area varies in industrial landscape causing school proximity to major areas of traffic to differ. Included in this initial sampling were soils from school areas which showed the overall highest levels of cPAHs and benzo[a]pyrene (B aP). Because schools in this metropolitan range tend to have a high degree of concern, there is the potential for oral exposure to PAHs in sensitive age groups by way of hand-to-mouth actions involving soil. Additional preliminary data from playground soil at elementary schools throughout the Oklahoma City Area have shown elevated but varied cPAH levels. These sites should be further investigated to determine potential sources of contamination.

### RP059 Potential risk of biomass-derived biochars as soil amendments

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Biochar is gaining increasing attention agricultural and environmental fields due to its high surface reactivity for contaminant immobilization and strong ability for carbon sequestration. Various agricultural wastes including swine manures and sewage sludge were used as biomass feedstocks to produce biochars, which was suggested as a promising method to utilize the wastes. Unfortunately, the potential risks from biochars have not been well-documented.This work reported potential risk of maize straw- and swine manure derived biochars pyrolyzed at 300 and 500°C. During the pyrolysis processes, total heavy metals in the biochars were enriched greatly accompanying with considerable emission of the heavy metals into atmosphere and the trends became increasingly obvious at high pyrolysis temperature. Meanwhile, the biochars showed significant changes in the speciation of the heavy metals compared to raw feedstocks. The changes in the chemical speciation and matrix properties led to reduced availability of the heavy metals in the pyrolyzed products. The water- and acid-washing treatments could significantly increase the releasing risks of heavy metals from biochars into the environment. The biochars usually contained environmental persistent free radicals, which emerged strongly as a function of the aromatization of biomass feedstocks and could exert negative effects on the growth of plants and microorganisms. Electron paramagnetic resonance analysis confirmed that the environmental persistent free radicals were free from the influence of water-, acid-, or organic-washing of the biochars and could remain stable even after aged in soils for 30 days. Dissolved biochars, the water-soluble fraction of biochar, showed distinct properties including lower molecular weight distribution while higher aromaticity compared to soil dissolved organic carbon. The dissolved biochars could act as both sorbent and carrier for contaminants and thus increase greatly the risk of mobilization and transportation of the associated contaminants in the environment. This study explore the transformation and occuring mechanisms of the materials with potential risk in biochars. The results of this study provide important perspectives on the possible risks and the safe usage of biochars as soil amendments.

**RP060 Recommendations on the Use of Existing Toxicological Data/ Information for Evaluating Noncancer Hazards of Uranium at Mining Sites**

*D. Llee, Arcadis In; B.H. Magee, ARCADIS; D. Pfeiffer, Arcadis U.S., Inc.*

The current chronic oral reference dose (RfD) for uranium (0.003 mg/kg/day) listed in USEPA's Integrated Risk Information System (IRIS) is based on a lowest observed adverse effect level from Maynard and Hodges (1949). USEPA (2000) used a study by Gilman et al. (1989) for derivation of a Maximum Contaminant Level (MCL) for uranium. In 2002, the USEPA IRIS program reviewed the RfD based on the Maynard and Hodges study and other available studies, including the Gilman study, and did not revise the IRIS RfD. ATSDR (2013) has recently developed an oral intermediate minimum risk level (MRL) of 0.0002 mg/kg/day for uranium (soluble salts) based on the Gilman study. Lastly, in 2016 the Superfund Technical Support Center recommended that the published IRIS RfD be rejected and the ATSDR MRL be used for risk assessment purposes. The Gilman study has serious shortcomings primarily because identified effects do not reflect a dose-response relationship which is a requirement for determining that an observed effect was due to treatment versus chance. In *A Review of the Reference Dose and Reference Concentration Processes* (USEPA 2002), USEPA states that a study used for RfD derivation should establish a dose-response relationship, and the shape of the dose-response curve should be consistent with the known toxicokinetics of the compound. Furthermore, in addition to being statistically significant, the effects used for RfD derivation also need to be biologically significant. The Gilman study does not meet any of these criteria. The uranium oral RfD is currently in Step 1 of the IRIS program review process, so a revised RfD from USEPA is not expected for some time. In the interim, risk assessors have to decide whether to use the existing IRIS RfD, the ATSDR MRL (used to develop RSLs), or a *de novo* RfD that they derive and support with adequate documentation. This presentation will perform a critical review of current toxicological reference values, discuss specific deficiencies in the Gilman study, demonstrate the inability to derive a benchmark dose using the USEPA (2014) Benchmark Dose Software program, and make recommendations as to how to assess the noncancer risks posed by uranium.

**RP061 Risk Assessment of Metals in Bulk and Fine Fraction Mine Waste Soil**

*J.A. Kountzman, Black & Veatch / Special Projects*

The U.S. Environmental Protection Agency currently recommends sieving soil with a #100 sieve to obtain a fine fraction (< 150 µm particle size) for assessment of human health risk due to incidental ingestion at Lead Sites. This recommendation is based on a review of particle size distribution of metals in shooting ranges, incinerators, mine tailings and background soil samples from mining sites and urban soils that demonstrated consistent enrichment in particle size fractions smaller than 150 µm (EPA, 2016). Furthermore, EPA recommends that the fine fraction be used for determining site-specific bioavailability and site-specific background. As part of an investigation at a historic mining area, 110 co-located bulk and fine fraction mine waste soil samples were collected and analyzed for arsenic, barium, cadmium, chromium, cobalt, copper, lead, silver, vanadium, and zinc. The data from the coarse and fine soil fractions were used to calculate the relative percent difference (RPD) between the two sets of data. In addition, regression analysis of the paired concentrations of each metal was performed to determine the nature of the relationship for the mine waste soils. A summary of the analytical results, the RPDs between the bulk and fine fractions, and regression analysis are presented for each metal. The degree of enrichment in the fine fraction was determined to be metal specific. The regression equations and trendlines demonstrate that although some sample pairs show evidence of enrichment for some metals, the data predominantly indicates that the concentrations in the fine fraction tend to be lower than the concentration in the coarse fraction. Since the fine fraction is more representative of the concentration available to both human and ecological receptors, use of coarse sample results may overestimate exposure and risk for the site. Recommendations for incorporating metal enrichment data in risk assessments of mine waste sites are presented and discussed.

**RP062 Development of an area-specific bioavailability factor for assessing human exposure-risk to arsenic in soils in southeastern Idaho**

*D. Pfeiffer, Arcadis U.S., Inc.; D. Llee, Arcadis In; B.H. Magee, ARCADIS; M. Hay, ARCADIS Inc.*

Cancer risks associated with exposures to arsenic via soil ingestion often drive human health risk assessments and understanding the range of arsenic bioavailability in soil at a site is important to avoid the overestimation of site-related human health impacts and unwarranted remediation costs. The United States Environmental Protection Agency currently recommends a default relative bioavailability factor of 60 percent for ingestion of arsenic in soils based upon the highest estimated RBA value from 11 hazardous waste sites using the juvenile swine method and summarize the relationship between the form of the arsenic and high, medium and low bioavailability. In this poster, the geochemistry at a site in the western US is presented to show that arsenic in the soil environment is most likely present as arsenate and/or arsenite strongly associated with iron oxyhydroxides rather than as a pure arsenic phase such as arsenic trioxide or arsenic sulfide. The high concentrations of iron compared to arsenic at the site also demonstrates an abundance of iron for adsorption and/or coprecipitation of arsenic, which is consistent with the association of arsenic with iron oxyhydroxides. This geochemistry is then tied to available USEPA studies to derive a site-specific relative bioavailability factor of 45 percent to reduce uncertainties associated with arsenic risks from soil ingestion.

**RP063 As, Cd and Pb Bioavailability in Contaminated Soils: Application to Soil Remediation**

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When considering metal exposure to humans via incidental ingestion of soil, daily intake will not only be influenced by their concentrations in soils but also by their bioavailability. While different animal models and feeding schemes have been used to assess metal-RBA, comparative studies detailing the influences of these parameters on metal-RBA measurement are lacking. In addtion, time, cost and ethical considerations limit use of in vivo assays. Therefore, in vitro assays have been developed to determine the amount of metal extracted from soil matrix in simulated human gastric and intestinal fluid, i.e, bioaccessibility. However, to determine if these assays provide a good prediction of metal bioavailability in soil, it is important to establish the relationship between in vitro bioaccessibility and in vivo RBA. However, established IVIVCs are often soil specific, varying with the soils used. Furthermore, to date, no study tested the feasibility of in vitro assays to predict metal-RBA in contaminated soils from China. The objectives of this study were 1) to compare metal-RBA in contaminated soils determined by use of different animal models to determine how in vivo operational parameters impact RBA determination; 2) establish IVIVC for metal in contaminated soils from China by correlating bioaccessibility determined using in vitro assays to RBA measured using in vitro mouse bioassays. The importance of including metal-RBA for refining metal exposure assessment and modifying soil cleanup standards for contaminated soils will be discussed

**RP064 Risk Assessments at Closed Small Arms Shooting Ranges Need to Consider Lead in Soil and in Lead Bullet and Shot Sources**

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Current procedures for evaluating lead at shooting ranges may miss lead in bullets and shot. Lead bullets/fragments/shot account for most of the lead remaining at shooting ranges which continue to weather to more soluble lead compounds in a site-specific manner (Rooney and McLaren 2000; Rooney et al., 2007). Each 150 grain, .30 caliber bullet initially contains ~8000 mg lead. The complete release and mixing of lead from one .30 caliber bullet would bring 100 kg of soil to the 80 mg/kg lead screening level for residential use in California. However, there is only about a 1 in 100,000 chance the bullet would be analyzed using grab sampling because EPA Method 3050B/6010B only digests 1 gram soil. This is not sufficient to assess risk of future use, since over 99.9% of the samples would miss the bullet and underestimate total lead and future lead exposures. We developed a screening-level survey approach that allows a metal detector to rapidly screen the top 6 inches of soil for density of lead bullets that would exceed 80 or 400 mg/kg total lead after complete bullet dissolution. Lead bullets were not visible at several shooting ranges that had been closed for 40 to 70 years, but were easily detected in the top 6 inches of soil with a suitable metal detector. Screening rifle and pistol ranges with a suitable detector easily identified many locations with a high density of lead bullets and over 10,000 mg/kg total lead that had been missed by gridded sampling for lead using X-ray fluorescence (XRF). This approach enhances initial screening of shooting ranges to help determine the lateral extent of lead bullets/shot. Combining this approach with measuring lead in fine soil, enables a more accurate estimation of total lead and future lead exposures. Thus, shooting ranges should also be screened for density of lead bullets/shot with a suitable metal detector. For detecting No. 9 lead shot, a different detector was suitable at up to 2 inches and could also aid in screening skeet ranges for regions of lead shot deposition and potential bird ingestion exposure. To account for total lead, we recommend collecting large samples where spent ammunition is most concentrated, sieving the entire sample to recover and quantitate lead bullets/shot gravimetrically. Lead should also be measured in fine sieved soil by XRF or EPA Method 3050B/6010B. Cleanup and risk management decisions regarding future use should be made on total lead in combined lead bullets/shot and fine soil.

**RP065 Recreational Exposure to Game Species Based on Habitat and COPC Soil Concentration**

*T. Biksey, C. Peterson, EHS Support LLC / Risk Assessment*

A preliminary conceptual exposure model (CEM) for a northwestern aluminum recycle facility shows the potential exposure to constituents of potential concern (COPCs) by recreational users (i.e., hunters). The site environment includes the facility operational area, landfills, and undeveloped areas. Extensive environmental sampling indicates variable distribution and concentration of COPCs across the site with higher concentrations associated with operational areas and landfills. Site human health exposure areas based on potential current and future land use includes commercial/industrial, residential, and recreational uses within the operational areas, landfills, and undeveloped areas. The terrestrial habitats associated with human health exposure areas that are uncovered consists of mixed conifer forest, riparian forest, deciduous shrubland, and open grasslands, and transitional habitats characterized by intermittent or seasonal surface water inundation that flow through the terrestrial habitats; these support multiple terrestrial receptor groups including game species. To assess the potential risks to the recreational hunter from the ingestion of game species foraging on the site, an exposure point concentration (EPC) of the COPCs is required to develop the dietary intake exposure model for the hunter using bioaccumulation factors. For game species with a wide-ranging foraging area (i.e., white-tailed deer), the exposure to COPCs will vary by the magnitude of the COPC-specific EPC in the soil of a specific human health exposure area, the habitat quality association with that exposure area, and the home range of the species. The habitat quality for each specific human health exposure area is a measure of species-specific habitat and life history characteristics. A GIS based web map provides the spatial scale for the habitat quality and COPC-specific EPC to estimate a site-wide weighted COPC-specific EPC for game ingestion exposure model. An example of the methodology using the white-tailed deer is presented. A sensitivity analysis provides a range of potential risks from variations in the area of future land use for recreational hunting.

**RP066 Iterative Evaluation of the Costs and Benefits of Determining Site-Specific Bioavailability: Case Study for Metal Grinding Dust**

*A. Verwiel, D. Proctor, M. Suh, ToxStrategies Inc*

Bioavailability is recognized as an important consideration in human health risk assessment because toxicity testing is conducted using forms of chemicals that are well absorbed, but chemicals in environmental matrices are frequently less available for absorption. Almost 30 years ago, Risk Assessment Guidance for Superfund suggested that bioavailability could be considered in human health risk assessment. But time, expense, effort and uncertainty surrounding regulatory acceptance, frequently outweighed the benefits of a more accurate assessment of potential health risks. With the advent of regulatory guidance (USEPA, 2007, 2017, ITRC, 2017) and the accumulation of scientific literature, bioavailability is a more accepted tool in risk assessment. Iterative assessment of the costs and benefits of quantitative bioavailability testing as data is gathered can facilitate the timely and effective integration of site-specific bioavailability into risk assessments. Based on recent experience with in vitro and in vivo bioaccessibility of nickel and cobalt from metal grinding, we have developed a road map for developing quantitative bioavailability applicable to other projects. This road map includes five steps: initial site characterization, in vitroassessment, further site characterization, pilot in vivo study, and comprehensive in vivo study. Practical and scientific considerations should be reviewed, such as regulatory acceptance and impact on potential remediation efforts, and solubility of metals in impacted media and the biological uptake of specific metals of interest. As the project progresses, the costs and benefits should be re-evalutated, such when the scope of remediation is further defined, changing the associated costs (up or down). Additionally, the results of in vitroand pilot in vivotesting may indicate that a full study would not sufficiently vary from the default of 100% or be too uncertain due to biological and kinetic factors. Incorporating input from clients, regulators stakeholders, and scientists through the course of the project is also important. For example, regulators who are unfamiliar with bioavailability testing may be unwilling to accept the results, eliminating most benefits of performing these tests. The road map outlined in this presentation provides a pragmatic approach for developing site-specific bioavailability for risk assessments.

**RP067 Pilot study on effects of U mining on cultural resources in the Grand Canyon watershed: Potential implications for Native American risk assessments**

*D. Cleveland, J. Hinck, USGS / Columbia Environmental Research Center*

The Grand Canyon watershed contains economically viable high-grade uranium (U) ore deposits in various stages of exploitation, yet the potential risks of U mining to Native American cultural resources are not well known. The staggered mining life stages and regional distribution of these deposits may result in prolonged exposure of biota, and subsequently to humans using the resources, to U mining-related constituents. Radiological concerns about U are often at the forefront of public concern, but U and other co-occurring elements may also pose toxicological risks. Previous work has indicated that aeolian transport of dust from the mine sites contributes to elemental-loading on vegetation beyond mine perimeters. We aim to develop a Native American risk assessment model for U mining, which will likely need to include additional considerations compared to a standard human health risk assessment. In this pilot study, unwashed sagebrush (*Artemisia tridentata*) collected from pre-production and post-mining locations, and from a nearby traditional cultural property, was burned to characterize chemical constituents in particulates under a simulated traditional use; thus evaluating inhalation risks. Particulate matter was vacuum-collected on a filter, and then analyzed for metals concentrations. Results from burned samples will be compared to particulates collected from unburned sagebrush, and to sagebrush collected up- and down-wind at and near the U mine sites. Results will also be used to determine whether further studies are needed to identify potential human inhalation risks associated with the cultural use of resources obtained near U mines.

**RP068 Health risk assessment of heavy metals from informal e-waste processing in Alaba International Market, Lagos, Nigeria**

*A. Adebayo, Federal University of Petroleum Resources Effurun / Environmental Management and Toxicology; O. Osibanjo, University of Ibadan / Department of Chemistry*

Informal electronic waste (e-waste) processing is a crude method of recycling, which is on the increase in Nigeria. The release of hazardous substances such as heavy metals (HMs) into the environment during informal e-waste processing has been a major concern. However, there is insufficient information on environmental contamination from e-waste recycling, associated human health risk in Alaba International Market, a major electronic market in Lagos, Nigeria. The aims of this study were to determine the levels of HMs in soil, resulting from the e-waste recycling; and also assess associated human health risk in Alaba international market. Samples of soils (334) were randomly collected seasonally for three years from four selected e-waste processing activity points namely; burning, dismantling, stockpiling and disposal. The samples were digested using standard methods and HMs analysed by inductive coupled plasma optical emission. Human health risk was done using hazard index (HI) for non carcinogenic elements and total cancer risk index ($R_{TOTAL}$) for carcinogenic elements. The concentrations of HMs (mg/kg) in soil samples from all studied areas showed that levels were several orders of magnitude higher than the background values. The following conclusions could be drawn from the carcinogenic and non-carcinogenic health risk assessment; the exposure pathway that results in the highest level of health risk was inhalation followed by dermal and lastly by ingestion. The hazard indices of Pb were above the threshold value of 1 for non-carcinogenic hazard risk indicating a possible risk of neurological and developmental disorder in children. The hazard indices for the four carcinogenic heavy metals Cd, Co, Cr and Ni were above the threshold range of $10^{-4}$ to $10^{-6}$ indicating a high risk of cancer in children and adults. The potential carcinogenic risk order with respect to e-waste processing activity is Burning> Dismantling> Disposal> Stockpiling. Generally, the levels of carcinogenic and non-carcinogenic risks were unacceptable being in excess of $1 \times 10^{-6}$ and 1 thresholds for $R_{TOTAL}$ and HI, respectively. Heavy metal contamination of Alaba international market environment resulting from informal e-waste processing was established. Proper management of e-waste and remediation of the market environment are recommended to minimize the human health risks.

**RP069 Assessment of the Environmental Pollution Effect of Lead Mining in Ebonyi State**

*A.I. Onwurah, University of Nigeria, Nsukka / Environmental Management; F.A. Atawal, University of Nigeria, Nsukka / Zoology and Environmental Biology*

The role environmental quality plays in human and animal health is unprecedented. In this work, we evaluated the health of the people, and plants in a community in Ebonyi State where mining activities had been on for over ten (10) years. Lead mining activities are the major sources of the contaminants The levels of contamination of bitter leaves (*Vernonia amygdalina*), ora leaves (*Pterocarpus mildbraedii*), scent leaves (*Ocimum gratissimum*), okra pods (*Abelmoschus esculentus*), curry leaves (*Murraya koenigii*) and garden eggs (*Solanum melongena*), soil and water , arising from the mining area in this community were evaluated. This becomes important as these plants as sources of food while water is used for different domestic purposes and even drinking. Toxicity of water samples were tested using the "ostracod (*Hetrocypris* incongruens) toxicity assay kit" and that of soil in earthworms. Results show that the concentrations the various vegetables, soil and water samples decreased as distances progressed from the mining site. There was no observed mortality of earthworms after 72hours of exposure to the various lead-soil samples. However, 10% mortality of earthworms (after 7 days) and 22.2% mortality (after 14 days) were observed in lead-soil samples collected 0.05km away from the mining site, while 10% mortality was observed (after 14 days) in lead-soil sample collected 2km away from the mining site. Percentage mortality of ostracod crustaceans exposed to lead-water samples decreased as distances progressed from the mining site, but remained constant after distance of 4km. Both Estimated Daily Intake (EDI) and Target Hazard Quotient (THQ) for lead in both adult and children for consumption of all the samples collected around the mining area decreased as distances progressed from the mining site. For some of the samples the EDI values were below the Tolerable Daily intake (TDI) for lead while for others, EDI values were above the TDI in both adult and children. Target Hazard Quotient (THQ) of lead in both adult and children for consumption of the various samples was greater (>) 1 for some of the samples and less (<) 1 for other samples. It is predicted that residents around lead mining sites are prone to health hazard and should therefore limit exposure. Also this result can help the local authority in planning for better management of these areas to ameliorate the negative health effect of the mining site on the community and map out environment recovery steps.

**RP070 Low concentrations of carbonaceous nanomaterials in soil affect soybean growth and dinitrogen fixation**

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With the increasing exposure of agricultural soils to carbonaceous nanomaterials (CNMs), terrestrial crop plants may be at risk. Published research, much of which has been conducted under hydroponic conditions over short-term periods, has shown a mix of positive and negative effects of CNMs on plants. However, long-term CNM effects on plants, including effects on plant-microbe interactions, are still relatively poorly understood. In this work, we determined the relative effects of two engineered CNMs (multi-walled carbon nanotubes and graphene) versus industrial carbon black on the growth and dinitrogen ($N_2$) fixation of soybean grown to bean production stage in soil. Each type of CNMs had three concentrations (0.1, 100, and 1000 mg $kg^{-1}$ dry soil). Plants grown with the lowest concentration of carbon nanotubes were shorter and had a lower leaf area at harvest. However, all three CNMs—at all concentrations—accelerated plant flowering. Root nodulation and $N_2$ fixation potential appeared negatively affected by CNMs, with stronger inhibitory effects more frequently occurring with the lower concentration CNM treatments. This inverse dose-response relationships were explained by the greater CNM agglomeration at higher concentrations which likely decreased CNM dispersal and bioavailability in soil, and thereby decreased the effects on plants. Overall, our work shows that low concentrations of CNMs in soil could have relatively high impacts on soybean growth and $N_2$ fixation. The discovery that CNMs can show inverse dose-response relationships means that CNMs may cause damage to ecosystems at concentrations lower than we might have otherwise assumed. Additionally, this highlights the importance of the processes that control CNM agglomeration and the stability of the agglomerates in regulating the overall effects of CNMs on terrestrial ecosystems.

**RP071 Ecological risk assessment for the PAHs-contaminated soils using a luminously modified freshwater bacterium**

*H. Keum, Korea University / College of Life Sciences and Biotechnology*

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds that are manufactured in the industry. Another major source of PAHs is the incomplete combustion of organic material such as coal, oil, and wood. PAHs are commonly exist in air, soil, and water. Thus, they are considered ubiquitous in the environment (Zhang and Tao, 2009). PAHs in the soil will be bound to soil particles so that they can move (Masih and Taneja, 2006). PAHs are highly lipid-soluble and also very soluble in organic solvent. The water solubility of PAHs decreases for each additional ring (Wilson and Jones, 1993; Haritash and Kaushik, 2009). The toxicity of PAHs is due to the formation of reactive metabolites. It has been proven that PAHs have carcinogenic and mutagenic effects (IARC 2010). Especialy, benzo(a)pyrene (BaP) is the first chemical carcinogen to be discovered and known to cause cancer in animals (USEPA 2008). Soil samples were taken from the clean site of a mountain (Hongcheon, South Korea) and air-dried and sieved through a 2 mm sieve. Optimum doses of Hb and $H_2O_2$ for degradation and detoxification of pyrene in soil samples were obtained from previous studies (Keum et al., 2017a; Keum et al., 2017b). The Hb concentration of 0.010 g (g soil)$^{-1}$ and $H_2O_2$ concentration of 0.03 g (g soil)$^{-1}$ were employed. The toxicity of uncontaminated control soil were compared with those of pyrene- or BaP-contaminated soils. Soils were contaminated with pyrene or BaP at a concentration of 10 mg kg$^{-1}$ and was treated with phosphate buffer (pH 7.0) for 24 h. Then, the soil was extracted and analyzed to measure EC50 concentration. After remediation of organopollutants in soil, it is necessary to estimate the toxicity extents and the effect on microbial community. Many previous studies have reported the remediation of organopollutants with the use of Hb and $H_2O_2$. However, until now, almost no attention has been given to the estimation of correlation between degradation progress and biotoxicity extent during the remediation with the use of Hb and $H_2O_2$. Here I have reported the comparison of toxicity with two bioluminescent bacteria *V. fischeri* and *J. lividum* YH9-RC during the remediation of pyrene and BaP. The bacterium *V. fischeri* has been employed for a long time to estimate a toxicity of organic compounds. However, in the present study, I have shown that the other bacterium *J. lividum* YH9-RC might be a better choice to estimate the change in toxicity of pyrene and BaP in soil.

**RP072 Back Conversion from Methyl Triclosan to Triclosan in Soil-Earthworm System**

*X. Du, University of California, Riverside; Q. Fu, Eawag, Swiss Federal Institute of Aquatic Science and Technology / Department of Environmental Chemistry; J. Gan, University of California, Riverside / Environmental Science*

Numerous pharmaceuticals and personal care products (PPCPs) pass through wastewater treatment plants (WWTPs), where biological and chemical treatments often result in the formation of a wide range of transformation products via reactions such as conjugation and alkylation. When wastewater irrigation or biosolid application occurs, such transformation products may be released into soil and taken up by soil organisms such as earthworm. In the present study, the high-volume antimicrobial triclosan was used as a model compound to investigate the back conversion from metabolites to parents. After 14-day exposure to environmentally relevant concentrations of methyl triclosan (a primary triclosan metabolite), the parent compound triclosan was detected in the soil. In the methyl triclosan exposed soil-earthworm system, triclosan was found in both soil and earthworm samples with triclosan level of 0.012 and 2.0 µg/g dw, respectively. The bioaccumulation factors (BAF) of methyl triclosan and triclosan were 21.5 and 172 respectively. Consistent occurrence of triclosan in soil confirmed the transformation of methyl triclosan to triclosan in soil. Relatively high BAF values of triclosan showed that biotransformation of methyl triclosan very likely occurred in earthworm after uptake. The back and forth conversion of contaminants such as triclosan extends the environmental persistence and exposure of parent compounds. The occurrence and fate of transformation products should be taken into consideration when assessing environmental risks of emerging contaminants.

**RP073 Those crazy kids: Is juvenile soil invertebrate avoidance to contaminated soil more sensitive than adults?**

*A. Gainer, University of Saskatchewan / Toxicology Centre / Soil Science, Toxicology; R. Akre, University of Guelph; S. Siciliano, University of Saskatchewan / Department of Soil Science*

Avoidance tests are simple toxicity tests with field and ecological relevant findings. Currently, the majority of soil invertebrate avoidance testing is conducted on adults, with no literature on juvenile avoidance responses. Existing toxicity literature in aquatic and soil environments agree, juveniles are more sensitive to toxicants than adults. In this study, our objective was to determine if juvenile avoidance response was more or less sensitive than the adults. We tested the dual avoidance response of adult and juveniles of three standardized soil invertebrates (*Folsomia candida, Enchytreaus crypticus* and *Eisenia fetida*) to three soil contaminants (phenanthrene, copper and sodium chloride) in artificial soil. Interestingly, we found the juvenile avoidance response was both more and less sensitive than adults. In addition, non-avoidance was observed by both juvenile and adult *Folsomia candida* and *Enchytreaus crypticus* when exposed to phenanthrene contaminated soils.

**RP074 Does the springtail *Folsomia candida* avoid free or total contaminants?**

*C. Teron, University of Saskatchewan Toxicology Centre; A. Gainer, University of Saskatchewan / Toxicology Centre / Soil Science, Toxicology; S. Siciliano, University of Saskatchewan / Department of Soil Science*

Avoidance toxicity tests are simple, rapid tests with ecological relevance. The avoidance response of soil invertebrates will vary depending on the exposure media it detects the contaminant, if it is detected at all. Thus, the avoidance response is largely linked to amount of free contaminant, whether it is in the soil gas phase (for volatile contaminants like some polycyclic aromatic hydrocarbons) or soil water phases (for heavy metals). The objective of this study was to assess the avoidance response of the standardized toxicity test soil invertebrate, *Folsomia candida*, to different levels of free contaminants. To vary the degree of free contaminants, we modified the peat content of artificial soil to 10% and 5%. Test contaminants included heavy metals (copper, cadmium), polycyclic aromatic hydrocarbons (phenanthrene, naphthalene and pyrene) and the standard soil toxicity test contaminant boric acid. The amount of free contaminants in metal contaminated soils was assessed using the Free Ion Activity Model and for polycyclic aromatic hydrocarbons contaminated soils activity was assessed using Fugacity.

**RP075 Quantification of Nano Zero-Valent Iron in Soil for Toxicity Testing**

*R. Akre, University of Guelph; W. Martin, B.A. Hale, University of Guelph / School of Environmental Sciences*

Nano zero-valent iron (nZVI) has been used for over twenty years in the remediation of groundwater and, more recently, soil. It can reduce the toxicity of contaminants such as polycylic aromatic hydrocarbons, pesticides, and heavy metals that are resistant to microbial degradation and weathering through degradation and immobilization. Before nZVI can be widely used in soil remediation efforts, toxicity testing must be done to compare the risk of the existing contaminants to the risk of the nZVI. Some literature exists on the toxicity of nZVI to soil biota, but these studies report nominal concentrations or reduction in bioactivity of a contaminant rather than measured concentration. This research endeavours to adapt a method of nZVI quantification from groundwater research that uses indigo disulfonate as a specific chemical redox probe. A standard curve is developed by reacting indigo disulfonate with known concentrations of nZVI to produce a colour change measured by ultraviolet-visible spectroscopy. This curve is then used to quantify nZVI in soil pore water extracted from nZVI amended soils. Toxicity testing on two species of soil invertebrates, *Folsomia candida* and *Eisenia fetida*, is then completed and combined with the method of measuring nZVI to express toxicity thresholds in terms of the realized dose in the soil.

## Novel Approaches for Understanding Diversity in Species Sensitivity to Chemicals

### RP076 A Multispecies Approach to Toxicity Testing With Nematodes

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Species differ across many levels of biological organization – each of which can affect how species respond to chemical exposure. Due to challenges in animal husbandry and resource availability, it is challenging to test many species in the lab at the same time to determine which aspects of species biology drive toxicity. Here, we show that multiple species of nematodes can be maintained and used for chemical testing with minimal resource input compared to many other types of organisms. Specifically, we tested several neurotoxic pesticides on four nematode species: *Caenorhabditis elegans*, *C. briggsae*, *Pristionchus pacificus*, and *Oscheius tipulae*. This model could be further expanded to include more – perhaps dozens – of additional species.

### RP077 High-Resolution Mass Spectrometry-based Metabolomics for Translating Exposure Effects Across Species

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Chemical exposures often pose dramatically different health risks in fish depending on the species being investigated. These disparities are frequently due to differences in metabolism, excretion, exposure route, and a host of other species-specific traits. However, due to time and resource limitations, toxicity studies are often restricted to a small subset of species, and thus may fail to capture all potential risks. Unfortunately, many current approaches for measuring adverse effects are not designed for making evaluations across species. Among the ‘omics approaches, metabolomics is particularly well suited to cross-species assessments, because it does not require genome sequences. Furthermore, in addition to evaluating impacts on endogenous biochemicals (the metabolome), metabolomics is increasingly being used to monitor anthropogenic contaminants in exposed organisms, including their metabolism and excretion, thus expanding its use for understanding exposures as well as effects. Finally, a variety of biological matrices that can be sampled non-lethally contain rich metabolomic information allowing for: the repeated sampling of individuals, the use of larger sample sizes to better evaluate resident populations and the study of threatened or endangered species, for which lethal sampling is forbidden. Here we report the use of high-resolution mass spectrometry-based metabolomics to explore effects of exposure to the model aromatase inhibitor, fadrozole, in four fish species: Japanese medaka, fathead minnow, zebrafish and mosquitofish. As an initial focus, results of skin mucus are presented, reporting the measurement of distinct metabolomics profiles across these four species and the complex molecular processes observed in response to fadrozole exposure. In addition, skin mucus samples from other phyologenetically-diverse fish species were evaluated to determine the potential for using non-lethal metabolomics to monitor environmental exposures in highly disparate species. Easily detectable and species-specific metabolomic profiles were detectable in the skin mucus across the species analyzed providing strong evidence that species-specific adverse effects can be monitored using this metabolomics approach. As a whole, these investigations demonstrate that metabolomics responses may provide unique perspectives into cross-species exposure sensitivity, the molecular basis for this diversity and important non-lethal approaches for making such determinations.

### RP078 Metabolic fingerprint of pesticide exposure in fish to determine biological basis for species sensitivity

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Pesticides TFM and niclosamide are used to control the population of sea lamprey (*Petromyzon marinus*), an invasive fish species in the Great Lakes. However, non-target species such as lake sturgeon (*Acipenser fulvescens*), are affected under certain conditions by these pesticides. A lower detoxification capacity by glucuronidation can partially explain the selectivity of TFM to sea lamprey when compared with other fish species. However, lake sturgeon is also susceptible to TFM despite being able to detoxify it through glucuronidation. To elucidate the biological basis for sensitivity variation across these two fish species, we focused on metabolism to determine the mechanisms of toxicity. We hypothesized that exposure to TFM or niclosamide (or their mixture), even at sub-lethal levels, triggered biological perturbations that generated specific groups of characteristic metabolites, or metabolic fingerprint, in sea lamprey and lake sturgeon. In order to test this hypothesis, sea lamprey and lake sturgeon were exposed to two concentrations of TFM and niclosamide, one lethal and one sub-lethal. Fish head and gills homogenates were extracted by liquid-liquid extraction with a mixture of methanol, acetonitrile, and water (2:1:1, v/v). Samples were analyzed by UPLC/HRMS in a Xevo G2-XS quadrupole time-of-flight (Q-TOF, Waters) mass spectrometer. Several metabolic features that were differentially expressed in control and exposed samples were selected for each species and further investigated. Sea lamprey metabolic fingerprint was compared to that of lake sturgeon to assess differential sensitivity caused by metabolic processes. This approach allowed us to have a better understanding of the mechanisms involved in the dysregulation of metabolic pathways by TFM and niclosamide to uncover endpoints of toxicity for both fish species.

### RP079 Quantifying Ecosystem Responses to Anthropogenic Stress: An Environmental DNA (eDNA) Approach

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To better understand ecosystem responses to anthropogenic stress, new biomonitoring methods are needed that assess biodiversity in a quick and cost-efficient manner. The emerging field of environmental DNA (eDNA) offers such an approach, as it can provide rapid, low-cost identification of the species in an area. To test the effectiveness of this new technology, eDNA will be collected from lakes along an acid-mine drainage (AMD) gradient within The Wilds Conservation Center (Cumberland, OH). Here there are several AMD-gradient watersheds, with each system often consisting of one AMD-affected headwater lake that filters downstream to several smaller lakes where pH returns to background levels. We previously observed fish recolonization downstream from AMD sources and plan to use eDNA sampling as a rapid, efficient tool to quantify biodiversity in fish, amphibian, and invertebrate communities along these AMD gradients. The goal of this proof-of-principle study is to estimate biodiversity and community structure at each lake using eDNA sampling and then compare these results with traditional ecological assessment methods (e.g., netting, electrofishing, invertebrate sampling). Water quality parameters (pH, metals, conductivity, dissolved oxygen) will also be assessed along the gradients for comparison to derived community structures. Our working hypothesis is AMD will reduce biodiversity and simplify community structure at lakes closest to the AMD source and that eDNA can be used successfully to detect these ecosystem changes. Results from this study will provide a wealth of species information beyond what is typically considered for remediation efforts. The extensive species data along a pollution gradient will allow for construction of site specific species sensitivity distributions (SSDs) for pH, metals, and other AMD characteristics. Knowing which levels of AMD correspond to desired community structures can help create AMD benchmark levels for any future attempts to restore affected lakes at The Wilds.

**RP080 Evaluation of microbial and chemical quality of ballast water collected from commercial ships entering Qatari waters**

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The Arabian Gulf countries including Qatar heavily depend on the transfer of oil and gas via commercial ships. Therefore, introduction of pollutants and invasive species possibly present in ballast water carried by these ships has become an emerging environmental issue in recent years. Although there are set of rules and regulations for discharge of ballast water into the Arabian Gulf, not all ships comply with these regulations. Since Qatar became a full member of the International Maritime Organization (IMO) in 2018, it is required that the regulations and policies of ballast water disposal should meet the chemical and microbiological quality standards. This project aimed at investigating the microbial and chemical quality of ballast water samples collected from commercial ships entering Qatari waters. The ballast water samples were collected from 20 different ships and tankers at Qatari ports. The physiochemical (pH, salinity, conductivity, and TOC) and chemical parameters (total anions and cations, as well as metals) of each sample were analyzed using different instruments. Additionally, microbiological analysis for total aerobic microorganisms, generic *E. coli* and total fecal coliform, and *Vibrio* spp. were conducted using selective media. The presumptive colonies grown on selective media were identified using 16S rRNA technique. The results show that out of 20 samples tested, 15 ballast water samples had total aerobic microbial counts above the set limit of 2 $Log_{10}$/mL. In terms of fecal coliforms and *Vibrio* spp., the counts were also above the set limits of 2.39 and 1 $Log_{10}$/mL, respectively. The preliminary results indicate that these samples need to be treated before their discharge into Qatari waters. Based on 16S rRNA method, *V. parahaemolyticus*, *Enterobacter hormaechei*, and *Pseudomonas* spp. were the major pathogens identified in ballast water samples tested. The results of chemical analyses demonstrated that majority of ballast water samples (40-80%) had high concentrations of Zn, Sr, Cu, and Li. A positive correlation between Mo, V, and Mg and the total aerobic microbial and *Vibrio* spp. counts was determined at $P< 0.01$. These findings demonstrate the urgent need to properly treat ballast water before discharging into the Qatari waters in order to protect the marine ecosystem.

## Bridging the Gap Between Toxicity Method Challenges and Regulatory Compliance for Effluent and Ambient Toxicity Programs

**RP081 Improving Communication Between the WET Laboratory and Regulatory Authority**

*N. Love, S. Skigen-Caird, GEI Consultants, Inc.*

The standardization of whole effluent toxicity (WET) testing through the United States Environmental Protection Agency's (USEPA) 2002 methods resulted in an easy to follow method for laboratories to provide as consistent data as possible. Despite an improved control of variability, WET tests still depend on living organisms to respond to stressors in consistent ways, which may not always occur. When it comes time to explain variability in WET tests, or to request a regulatory conclusion on WET data, oftentimes regulators rely on state-specific, over-simplified, and sometimes dated implementation guidance that may not consider the full complexity of WET tests. Despite the EPA's attempts to improve understanding of WET methods through videos and other training formats, many regulatory authorities are under-funded, and over-worked, leaving little time for them to learn the intricacies of a method that they have never seen in action. Some regulatory agencies are lucky enough to have a specialist dealing with WET, but this is generally the exception. For the regulatory authorities tasked with making far-reaching conclusions on WET results, communication between the WET lab and regulator is key. By strengthening the knowledge exchange between the research community and regulators, more appropriate decisions can be made, improving trust and accountability for all parties. A 10-point method has been developed to improve communication between WET laboratories and regulators and is presented here.

**RP082 USEPA's Current *Ceriodaphnia dubia* WET Method and Other Applications to Better Understand Toxicity to Aquatic Life at Complex Sites**

*N. Love, GEI Consultants, Inc.; S. Skigen-Caird, GEI Consultants, Inc. / Ecological Division*

The *Ceriodaphnia dubia* whole effluent toxicity (WET) test method was refined by the United States Environmental Protection Agency (USEPA) in 2002 to provide better reproducibility of the data to ensure appropriate regulatory decisions were made based on the results of these tests. In many cases, the improved method has led to a protection of the receiving streams that was not reached before; however, in some cases the method does not provide a complete picture of the potential impacts on aquatic life. In these cases, additional methods should be used to evaluate the effluent to provide a complete picture of the effluent's impact on aquatic life. When used in combination, methods such as chemical analyses, instream biomonitoring, ambient WET testing, toxicity identification evaluation (TIE) testing, and alternate test species/new WET methods can help provide a more complete picture than standard compliance WET tests alone. Our evaluation of compliance WET tests and numerous additional methods for two sites has led to a more complete picture than the compliance WET tests alone. These methods, when used in a weight of evidence approach can provide a more accurate method of site evaluation. Making these additional methods more openly available for site evaluation through various regulatory approval pathways results in additional data available for use to better protect the environment. The lessons learned and suggested regulatory implementation are discussed.

**RP083 Effects of season and LED light on *Ceriodaphnia dubia* culture board survival, fecundity, and neonate reference toxicity test performance**

*R. Cooper, Arkansas State University; J. Bouldin, Arkansas State University / Ecotoxicology Research Facility*

Most incandescent light sales will be banned by 2020, so alternatives for use in culture organism light/dark cycles need to be investigated. Although they have a slightly higher up-front cost, LED lights last longer, are cheaper to operate over time, and produce less heat than incandescent lights, which is important for maintaining stable culture and test conditions. Over two three-month periods (February-April and August-October), we tested if there was a difference in *Ceriodaphnia dubia* survival and fecundity, as well as neonate performance in acute and chronic reference toxicity tests (NaCl) from culture boards maintained with incandescent or LED lights with comparable light intensity. We compared survival, 14-d average total neonates, number of broods, days to third brood of 8 or more neonates, and number of days to first brood for culture boards. Endpoints for reference toxicity tests included LOEC, EC50, IC25, and control group variation. We found no relative differences between any parameters tested within each test period, but that *C. dubia* performed better overall during the the August-October period. Therefore, we conclude that LED lights at comparable light intensity are a viable alternative to incandescent for *C. dubia* cultures.

**RP084 Amelioration of Low Hardness-Induced Effluent Toxicity to *Daphnia pulex* by Restoration of Effluent Hardness**

*S.L. Clark, R. Ogle, Pacific EcoRisk; P. Worland, Los Alamos National Laboratory*

Several treated effluent discharges from Los Alamos National Laboratory (LANL) are regulated under NPDES permits that require acute *Daphnia pulex* toxicity testing. While the Los Alamos region is surrounded by surface waters categorized as very hard (>181 mg/L), it can be considered an "island" with lower surface water hardnesses, typically in the moderately hard (60-120 mg/L) and hard (121-180 mg/L) range. However, the source of water used at LANL is not the local hard surface water, but rather is domestic tapwater (high-quality groundwater from the local aquifers via three water supply well fields, each of which has different hardness ranging from 29-56 mg/L [as $CaCO_3$]). This tapwater is used in LANL's facilities for a variety of applications. The wastewater for one area using this tapwater is collected into an influent tank at a typical hardness of 40-45 mg/L. However, subsequent treatment processes reduce the hardness to approximately 0.2-12 mg/L. Our laboratory evaluated 24 acute toxicity tests for that particular outfall and determined that there was an increase in mortalities associated with extremely low hardness levels with virtually no toxicity being observed when the hardness was >25 mg/L, which is consistent with several published studies indicating deleterious effects on other cladocerans at 5–50 mg/L hardness. As the LANL outfall discharges to 'harder' receiving waters, we recommended that LANL implement measures to increase the hardness of the effluent to the original source water levels, as some regulatory agency guidelines similarly call for the restoration of water hardness levels to those concentrations existing prior to the application of treatment processes that remove $Ca^{2+}$and $Mg^{2+}$. In order to evaluate the efficacy of such an adjustment, we performed side-by-side *D. pulex* toxicity tests of effluent and effluent restored to a hardness of 40-50 mg/L and consistently observed improved survival in the hardness-adjusted effluent. This study demonstrated that low hardness conditions can be a test interference for *D. pulex* tests, and that hardness amendments to match original source water levels can mitigate the apparent toxicity.

**RP085 Effect of *Ceriodaphnia dubia* Culture Hardness on Laboratory Control Test Performance**

*S.L. Clark, A.M. Briden, Pacific EcoRisk*

The US Environmental Protection Agency (USEPA) freshwater chronic toxicity manual (EPA-821-R-02-013) acknowledges that the type of water used for organism culture and dilution water is extremely important, and should come from the same source. The manual recommends that synthetic, moderately-hard water should be used as the culture and dilution water for testing performed to meet NPDES permit objectives. However, this guidance does not address how culture conditions may affect the test organism response. For example, it is unclear if toxicity testing performed with organisms cultured in moderately-hard water would have comparable biological responses to those obtained from organisms cultured in soft water. In order to address the concern of comparability of test results, we performed a study evaluating the outcome of the chronic *C. dubia* testing performed with organisms cultured in different hardness waters. In addition to our standard moderately-hard (80-100 mg/L) culture, we established a soft water (40-48 mg/L) culture that was acclimated to this culture water for several weeks. We then performed paired dilution water performance assessments using organisms cultured in moderately-hard and soft water. The results of this testing are presented, and ramifications for NPDES compliance determination are discussed.

**RP086 Assessing Food Preparation and Storage as Sources of *Ceriodaphnia dubia* Culture Quality and Chronic Test Variability**

*K.N. Prosser, Pacific EcoRisk / Quality Assurance; A.M. Briden, S.L. Clark, B.C. Jorgenson, Pacific EcoRisk*

Multiple inter-laboratory variability studies have identified significantly different outcomes for split samples tested using the chronic *Ceriodaphnia dubia* reproduction and survival test method (EPA 1002.0). Similarly, the culture performance for this species can also result in highly variable intra-laboratory testing. This variability is troubling and can result in diminished confidence in the testing protocol by regulated parties. In an attempt to determine, and subsequently control, the source of this variability, our laboratory has observed that food is the primary driver for *Ceriodaphnia* culture health and overall test performance. We suspect that, since food is the primary driver, food may also be a large contributor to the intra- and inter-laboratory variability associated with this species. In fact, the method allows for a significant amount of latitude for food type and preparation; this latitude may be a key driver of inter-laboratory variation. What then is the source of intra-laboratory variation, assuming the same protocols are used to acquire and administer each subsequent batch of food? Our study aimed to assess the potential sources of culture downturns and diminished test performance as it relates to *Ceriodaphnia* food, with focus on the most common food types used, *Selenastrum capricornutum* and YCT (yeast, Cerophyl, and trout chow). These sources include, individual food components (Could using different components alleviate poor performance?), vendors (Do vendor protocols always produce the best quality food?), laboratory supplies (Have the supplies been cleaned appropriately?), and age/freshness of the food batch (Does storage impact food quality?). Our study suggests that improvements to the method are necessary including standardized food preparation guidelines, time allowance for thorough QC vetting of each food type before expiration, and clearer survival and reproduction benchmarks for culture quality. For *Ceriodaphnia* culturing and testing, an ounce of prevention may save you a pound of cure .

**RP087 Evidence of Pathogen Interference and Pathogen Focused Treatments for Testing with *Ceriodaphnia dubia***

*N. Lynch, S. Vasquez, S.L. Clark, Pacific EcoRisk*

The USEPA manual for chronic toxicity testing only briefly addresses pathogen interference in tests performed with *Ceriodaphnia dubia* but has a far more detailed description of pathogen interference for the chronic *Pimephales promelas* test. This phenomenon is typically characterized by a flat concentration response curve, high inter-replicate variability, and possibly observations of microbes on live organisms. If the observed response cannot be eliminated with a change in dilution water, or a modification to the test design, and the pathogen interference has been confirmed after performing a re-test, the manual states, "the regulatory authority may allow modifications of the effluent samples or receiving water diluent to remove or inactivate pathogens… the effects of pathogenic bacteria and the effectiveness of the selected pathogen control technique must be confirmed by parallel and simultaneous testing of the technique with altered and unaltered samples". Our evaluations consisted of performing the USEPA freshwater 3-brood (6-8 day) survival and reproduction test with *C. dubia* to evaluate the efficacy of different treatments for pathogen removal. A variety of sterilization techniques were evaluated for the removal of pathogen interference symptoms. Testing was performed with POTW effluent which consistently exhibited pathogen interference, as previously mentioned. Results of these evaluations are summarized and discussed in the context of efficacy for pathogen removal.

## Bivalves as Indicators of Exposure and Ecosystem Health

**RP088 Contaminants of emerging concern monitoring by Great Lakes Mussel Watch Program**

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The Great Lakes Mussel Watch Program utilizes a multi-matrix approach that deploys caged and in situ dreissenid mussels, polar organic chemical integrative sampler (POCIS), and semipermeable membrane devices

(SPMD) to spatially characterize the presence of contaminants of emerging concern in the rivers, harbors, connecting channels and offshore areas of the Great Lakes. By leveraging various resources to achieve common goals of CEC characterization, MWP investigated the occurrence and magnitude of a broad list of CECs in mussels collected from several Great Lakes locations with several land-use characteristics. Herein, we summarize the CEC data obtained between 2013-2017 and present the percent detections and magnitude in various matrices, and the spatial distribution of CECs with respect to land use. The results presented will serve as an initial assessment to characterize various suites of CEC that had the highest probability of being detected in tissue, and to identify what methods could be used in the future.

**RP089 Linear mixed model analysis of mussel metabolomics data**

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NOAA's National Centers for Coastal Ocean Science Great Lakes Mussel Watch Program (MWP) has been monitoring in the Great Lakes since 1992 using dreissenid mussels as indicator organisms. Through coordination with Great Lakes Restoration Initiative since 2010, the program expanded its monitoring activities in the region with the objective of providing data and information in making sound science based management solutions for the restoration of Great Lakes. The program incorporated newer approaches and techniques into its traditional chemical monitoring protocol to meet the evolving environmental challenges. Under the ongoing Great Lakes Action Plan II, MWP has initiated monitoring contaminants of emerging concern (CEC) and assessment of the effects of these emerging compounds on mussel health. A pilot study to monitor a broad suite of CECs and associated effects on bivalve health using metabolomics was undertaken in Maumee River in Lake Erie in 2016. Quantitative environmental metabolomics is an emerging high-throughput approach and requires systematic analysis of data to account for unwanted variation due to factors such as batch effects and confounding biological variation. Herein we present a multimodel inferencing approach using a linear mixed model analysis of mussel metabolomics data to identify significant differences in metabolomics concentrations among locations in the Maumee River. In addition, multivariate analyses of CEC data are used to characterize each location and link it to metabolomics data.

**RP090 Using metabolomics to connect exposure and effect in mussels**

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The Mussel Watch Program (MWP) has used bivalves to monitor contaminant levels and environmental health since 1986. In 2009, monitoring of legacy contaminants was expanded to the U.S. Areas of Concern to support the Great Lakes Restoration Initiative (GLRI). In 2015, Under Action Plan 2 of GLRI, MWP together with other federal partners began to address contaminants of emerging concern and explore the use of effects-based indicators. Bivalves, as generally immobile filter feeders that bioaccumulate contaminants, can serve as sentinel markers of both exposure and effect. The ability to observe bivalve metabolite profiles distinct to geographical locations and to combine this information with contaminant data provides a powerful tool to monitor this environment and to understand the biological impact of environmental changes. Understanding temporal effects in metabolome profiles can help inform exposure patterns and mussel biology. In this study, we analyzed the metabolomes of caged dresissenid mussels and clams at several great lakes sites including the Rouge, Detroit, and Maumee rivers (2016) and in the Milwaukee river (2017). A targeted metabolomics approach measured the concentrations of 222 metabolites including amino acids, biogenic amines, lipids, fatty acids, hexose and metabolites associated with energy pathways. Contaminant data for a large set of persistent organic pollutants and contaminants of emerging concern was also measured in mussels, and in the surface water. Multivariate and correlational statistical analyses were performed on the data to identify relationships between geographical sites and timepoints. Results from 2016 indicate that metabolite profiles could successfully reflect distinct site and species differences, and correlation analysis identified 94 contaminant measurements that correlated with 125 metabolites ($|r|>0.8$. Preliminary results from the 2017 collection time series experiments indicate that differences between sites can be seen as early as 5 days and consistently at 55 days. ANOVA indicated that metabolite changes show two patterns, early indicators of change which are observable by day 2, but that revert to the initial concentrations after more time, and those that change consistently, either up or down, between 2 and 55 days of exposure. Site-specific differences and biological significance of metabolite changes will be explored.

**RP091 Assessing genotoxicity in freshwater mussels in field and laboratory studies**

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A variety of chemicals can induce damage to the DNA of animals, which, if not repaired, can lead to a cascade of biological effects. First developed in the 1980s, the single cell gel electrophoresis assay – or Comet assay – is used to visualize and quantify DNA damage. In recent years, this approach has been used to evaluate DNA damage from exposure to specific chemicals, as well as from complex mixtures of waterborne contaminants, such as industrial or municipal wastewater effluents. DNA damage has been associated with reduced growth, abnormal development and reduced survival in a variety of aquatic animals; thus, the Comet assay could be used as a biomarker of exposure to conditions susceptible of increasing DNA damage in freshwater organisms. Over the last few years, we have developed the Comet assay in freshwater mussel hemocytes, for its application to field and laboratory studies: We completed field assessments of DNA damage Giant Floater mussels exposed to urban-derived contamination from southern Ontario watersheds, and where we observed a 35% increase in DNA damage in mussels from a heavily-urbanized site compared to an agricultural site. In a related study, we exposed mystery snails to sediments from Hamilton Harbour Area of Concern, contaminated with metals, PAHs, PCBs and dioxins. A 96-h exposure induced more than 35% increase in DNA damage in mystery snails *Cipangopaludina*, compared to control sediments. We performed several in vitro studies with data-poor priority chemicals (e.g., substituted phenyl amine antioxidants, naphthalene sulfonic acids), where we tested single chemicals using a combination of in vitro exposures with freshwater mussel hemocytes, and whole-mussel exposures. This research permitted to determine potential genotoxicity of priority chemicals. We assessed the genotoxicity of wastewater effluents and wastewater-influenced surface waters from large urban centres from southern Ontario, using in vitro laboratory studies, which showed that some wastewater effluents appear to contain genotoxic substances. The highlights of these studies will be discussed in the context of further relating DNA damage to other measures of organism health (e.g., tumours, growth, health), and potential applications for the development of water quality standards and procedures for the protection of aquatic life and ecosystem health.

**RP092 Determining freshwater mussel presence/absence in rivers using environmental DNA**

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Freshwater mussels are excellent indicators of water quality and are particularly sensitive to ammonia toxicity. In California's Central Valley, state adopted water quality objectives (state term that is equivalent to federal "criteria") do not include an objective for ammonia. Rather, the state permits discharges of ammonia from Publically Owned Treatment Works (POTWs) and other sources based upon USEPA's national recommended water quality criteria for ammonia. Upon USEPA's promulgation of the 2013 ammonia criteria, it became important for state regulators to determine whether freshwater unionid mussels exist in individual water bodies into which POTWs discharge in order to determine whether USEPA's 2013 national recommended or "unionid mussel absent" ammonia criteria would be applicable to each discharge site for permitting under the National Pollutant Discharge Elimination System (NPDES). Thus, to establish proper ammonia effluent limits in NPDES permits, state regulators have requested POTWs provide information on the presence/absence of unionid mussels in the waterbodies where they discharge treated effluent. Environmental DNA (eDNA) from water samples is a powerful and rapid tool for determining presence, and hence occupancy of freshwater mussels without harming the surrounding ecosystem or the mussels themselves. In this study we developed and validated a quantitative polymerase chain reaction (qPCR) assay for the 3 freshwater mussel taxa that currently exist in California's Central Valley. We then introduced a cage of 20 *Gonidea angulata* into a riverine environment and collected eDNA samples at evenly spaced downstream intervals to determine how habitat type and distance from the caged mussels affected eDNA detection. Using occupancy modeling we determined that higher detection probabilities occur in riffle habitats compared to pool or run habitats. We also found surface samples work well for collecting freshwater mussel eDNA in riffle habitats, but are less likely to capture mussel eDNA in pools or runs. The maximum downstream distance at which the eDNA of the 20 placed *G. angulata* mussels were detected was 8 km. Our study demonstrates that eDNA is a reliable tool for determining the presence/absence of freshwater mussels in riverine systems, but that sampling should consider habitat type and season for the most effective results.

**RP093 Environmental Metabolomics for in situ Monitoring of Eastern Oysters Affected by Stressors from Point and Nonpoint Sources Along Coastal Georgia, USA**

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In the wild, eastern oysters (*Crassostrea virginica*) routinely adjust their physiology according to the numerous stressors present in their environment. However, the underlying biochemical processes, at the level of the metabolome, that represent these responses are poorly understood. Thus, we utilized untargeted GC-MS metabolomics to assess the metabolome of native oysters collected from oyster beds at the Brunswick River in Brunswick, GA and small tidal creeks at Sapelo Island, GA. Adductor muscle (n=5/site/month) was sampled every two months at each site from October 2014 – August 2015 and changes in the relative abundance of endogenous metabolites were determined via ANOVA with Fisher's LSD post-hoc testing. Putatively identified metabolites were associated with cellular energetics (i.e. citric acid and glyoxylate cycles as well as fatty acid and carbohydrate metabolism), amino acids, the urea cycle, cellular signaling and nucleotide metabolism. During colder sample months, there was an increased reliance on fatty acids and a decreased utilization of carbohydrates and amino acids as a source of energy. This suggests that oysters, during these months, are utilizing their nutrient reserves as a primary energy source. Supporting this, there was a decrease in N-acetyl glucosamine levels, which via its role in bivalve cilia beating, implies that the oysters were feeding at a reduced rate. With increasing water temperatures, the oysters appeared to utilize more readily available sources of energy, as there were large increases in citric acid cycle intermediates, carbohydrates and amino acids. There was also a concurrent increase in the abundance of the secondary signaling molecules, inositol and myo-inositol. These biochemical changes coincide with the normal spawning period of oysters in Georgia and imply that reproductive processes drive large-scale metabolomic changes. Changes in abundance for carbamic acid (glucuronidation substrate), aminomalonic acid, aminoadipic acid and glutamate (oxidative stress response), between the sample sites and months at both estuaries suggests that there are spatial and temporal differences in the stressors present that could also adversely affect the oyster's metabolome. This research highlights the utility of untargeted metabolomics in quantifying how seasonal changes in water quality affect the metabolome of an estuarine bivalve along coastal Georgia.

**RP094 Microplastics in Pacific Northwest Bivalves? Determining organism concentrations and estimating human consumption levels**

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The Pacific Northwest (PNW) supports a diversity of shellfish species which have been integral to PNW natural history and cultural heritage for millennia. Indigenous peoples in Oregon and Washington have subsisted on razor clams for centuries, and they are now a commercial and recreational staple. As human influences on the environment increase, many stressors and threats to these iconic species have emerged in the PNW and around the world. Microplastics are plastics smaller than 5mm in size that are intentionally manufactured or broken down from larger plastic pieces (Gall et al. 2015). These tiny fragments, pellets, and filaments originate from both marine and land-based sources, and frequently enter waterways through careless disposal, marine dumping, fishing gear loss, pollution, runoff, and wastewater. Marine organisms mistake microplastics for food items, which become incorporated into their guts, respiratory organs and tissues (Akpan 2014; Cole et al. 2011; Von Moos et al. 2012). While microplastics have been observed on PNW beaches, concentrations in seafood items have not been well-documented in this area, until a study was initiated in Oregon in 2017 by Baechler and Granek to quantify the types, concentrations, anatomical loadings, geographic distribution, and seasonal differences of microplastics in Pacific oysters and Pacific razor clams in Oregon and Washington. In order to estimate the potential human exposure rates of microplastics from razor clam consumption in the Pacific Northwest, a written survey was also administered to clammers on the Olympic Peninsula, Washington in April 2018. It aimed to identify frequency of razor clam consumption, preparation type, and number of individual razor clams consumed by respondents of varying demographics. Our findings will enhance the PNW's capacity to understand whether microplastics are a threat to coastal marine organisms or the humans who consume them. Highlighting the issue of plastics in the environment, and in important food sources, may ultimately lead to action on the management or policy level to reduce plastic transmission into the ocean.

**RP095 Platinum bio-accumulates and affects anti-oxidative biomarker profiles in the Zebra mussel (*Dreissena polymorpha*) after short-term exposure**

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Platinum (Pt), one of the rarest elements on Earth, has excellent electrical conductivity and catalytic properties. Due to its exceptional characteristics, it has been widely applied in the automotive industry in catalytic converters and has also been utilized in the field of oncology due to its antineoplastic properties. Consequently, the wide-spread use of the precious metal has led to its increasing presence in the environment where the chemical composition of water and soil may alter its bioavailability and influence its uptake and accumulation in a host of living organisms – especially those found in the aquatic environments where pollutants tend to accumulate. Although previous studies have measured the presence of Pt in the natural environment, comprehensive characterisation of possible adverse effects following exposure of organisms to the metal remains elusive. To address this, a bivalve was identified as a relevant model to assess the accumulation of Pt and its subsequent biological effects in freshwater organisms. Zebra mussels (*Dreissena polymorpha*) were exposed to a range of Pt concentrations (0.1, 1, 10 100 and 1000 µg/L) for four days, after which bioaccumulation was measured and compared to alterations in biomarker profiles relevant to metal toxicity i.e. glutathione-S-transferase, catalase (CAT), malondialdehyde (MDA) and metallothionein (MT) content. During Pt exposure, bioaccumulation occurred in muscle soft tissue, accompanied by a decreased Pt concentration in the exposure media due to both biological and non-biological processes. At concentrations of >10 µg/L, Pt induced increased activity of antioxidant enzyme systems (GST; CAT) and a time-dependent trend of increased lipid peroxidation. Bioaccumulation of Pt was also associated with a concentration-dependent increase in Pt-MT, thereby offering support to anti-oxidative pathways by removing free reactive Pt. Although these effects occurred at levels higher than those present in the environment, it indicates that Pt has the ability to cause aberrancies in metal-associated biomarker profiles even at low concentrations following short-term exposure.

**RP096 Adverse reproductive impacts of multi-metal contamination on oyster *Crassostrea hongkongensis* in the Pearl River Estuary, China**

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As the largest estuary in Southern China, the Pearl River Estuary is seriously impacted by trace metal pollution as a result of rapid urbanization and industrialization. Despite of much evidence of metal contamination, little is known about the impacts on local marine life in this estuary. In the present study, the reproductive state of four populations of oyster Crassostrea hongkongensis, a dominant species in the Pearl River Estuary, was for the first time examined based on a one-year field study. The levels and types of metal accumulation in the oysters varied greatly among different populations. In particular, oyster populations from the more contaminated sites had a poor reproductive state in comparison with those from the relatively clean sites, as manifested by a lower gonad condition index, a decrease of gonad cover area, and more importantly, an imbalance of the sex ratios. The concentrations of Cu, Zn, Ni, Co and Pb in the gonad of the more contaminated oysters were much higher than those of the relatively clean oysters. Especially, gonad somatic index and gonad cover area of oysters during the breeding season were significantly and negatively correlated with the levels of gonadal metal accumulation. Our results indicated an adverse impact on the reproductive health of local oyster populations in a field environment associated with long-term multi-metal exposure.

**RP097 Baseline tissue concentrations of trace metals in three common bivalves in the Chinese coastal waters and worldwide**

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Biomonitoring in aquatic system is one effective strategy to assess the potential risks of metal pollution and toxic effects. Bivalves have been extensively employed as biomonitors/sentinels of coastal pollution since the 1970s. Data mining of metal concentrations in the bivalve soft tissues needs to delve the deeper information and comparability in specific species and geographic environments. To explore the large-scale spatial pattern and baseline concentration of bioavailable metals, we analyzed the trace metals (including Cd, Cr, Cu, Ni, Pb, and Zn) in the soft tissues of oysters, mussels, and clams from different continents. Compared with the world historical metal databases, the median concentrations of Cd, Cr, Cu, and Zn in oysters along the Chinese coastal waters in 2015 were higher. However, the concentrations of Pb, Ni in oysters, as well as metals in mussels and clams were all lower than the world historical records. By conducting metal concentration frequency analysis, we modeled the baseline metal concentrations in these bivalves. We further modeled and checked the potential baseline concentrations of metals in these bivalves using a developed biokinetic/biodynamic model. Our comparisons suggested that the baseline concentrations of Cd, Cu, and Zn based on the concentration distribution frequency were comparable to those predicted by the biokinetic/biodynamic model. Our study provided a much more innovative method to establish the baseline metal concentrations in bivalves and biota environmental quality standards for the coastal management, biomonitoring, geochemical records/notes in the world.

**RP098 Physiological Response of Amblema plicata to Contaminants in the Guadalupe River Basin, Texas**

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Aquatic pollution has contributed to the significant decline of unionid mussel populations throughout North America. In this study, adult threeridge mussels (*Amblema plicata*) were collected from 8 sites within the Guadalupe River basin and the concentration of 16 trace elements, including As, Cd, Cu, Hg, Se, and Zn were determined in gill and foot tissue using microwave acid digestion and ICP-MS analysis. In addition, water samples were also collected at each site to determine the concentration of nutrients (total nitrogen, total phosphorous, ammonium) and *E. coli* at each site. The physiological response of mussels to contaminants was quantified through biomarker analysis (lipid peroxidation, antioxidant capacity against peroxyl radicals, and total protein concentration) of gill tissue. The concentration of As, Cd, Cu, Se, and Zn was higher in the gills than in the foot at all sites, whereas Hg was higher in the foot at upstream sites and not significantly different from the gills at downstream sites. The Se:Hg molar ratios across sites ranged between 8 and 24 in the gill and 4 and 11 in the foot, indicating that Se may have a protective role against Hg toxicity. On average, Zn was found at highest concentration in gill tissue (395 to 488 µg/g dry wt), followed by As, Cu, Se, and Cd, and Hg was found at lowest concentration (0.061 to 0.187 µg/g dry wt). There was no significant difference in the concentration of Zn in gill tissue between sites, whereas there was a significant difference for As, Cd, Cu, Hg, and Se. There was no relationship between trace element concentrations and investigated biomarkers at each site, with the exception of a positive correlation between Co and Cd concentration and lipid peroxidation. Higher nutrient concentrations at upstream sites correlated with a) higher lipid peroxidation and lower antioxidant capacity against free radicals, indicating greater oxidative stress, and b) lower protein concentration showing reduced overall health. This is the first in-depth study on the impact of environmental contaminants on unionid mussels in Texas rivers and the findings conclude that mussels are exposed to a mixture of urban and agriculturally-derived contaminants.

**RP099 Sublethal toxicity and metal bioaccumulation of Corbicula fluminea near a coal-ash effluent**

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The storage of coal combustion residuals (CCR) in aquatic surface impoundments has been a common practice across the United States for decades. Typically these aquatic surface impoundments are designed to allow the upper portion of the ash-basin water column to be removed and emptied into nearby freshwater or coastal systems. This method of coal-ash storage and disposal creates the potential for chronic point-source inputs of toxic metals into vital aquatic resources. The goal of this study was to investigate the potential ecological impacts of coal-ash associated metals on a freshwater ecosystem adjacent to a decommissioned coal-ash basin on Mountain Island Lake, NC. Ecological impacts were quantified through chemical analysis of water and sediments, and the use of the freshwater clam *Corbicula fluminea* as a bioindicator species. Clams were allocated into cages and deployed in situ at five sites near the ash basin for a one-month duration. Site water and sediments were collected from each study site and analyzed for common coal-ash associated metals and particle size composition. One-week laboratory exposures were also conducted using field collected water and sediments. Study sites included two locations within Mountain Island Lake's coal-ash effluent mixing zone, two locations further downstream, and one upstream reference site. After exposure, clam soft tissues were assessed for coal-ash associated metal bioaccumulation using Atomic Absorption Spectroscopy (AAS) and clam health was assessed using a suite of sub-lethal cellular toxicity assays (lysosomal destabilization, lipid peroxidation, and micronucleus.) The use of these integrative methods can provide important insights into the temporal and spatial scales of biological toxicity of CCR associated toxicity while also serving to further validate *C. fluminea* as a suitable bioindicator species in freshwater systems.

**RP100 Long-term effects of zinc and a zinc-lead-cadmium mixture in water on juvenile freshwater mussels**

*J.L. Kunz, J.M. Besser, N. Wang, J.A. Steevens, USGS / Columbia Environmental Research Center*

Field studies have found that the abundance and diversity of freshwater mussels were reduced in metal-contaminated streams that drain the Tri-State Mining District (TSMD) of Missouri, Kansas, and Oklahoma. However, previous 4-week laboratory toxicity studies indicated that juvenile mussels were not among the most sensitive benthic taxa. We recently conducted a series of longer-term (12-week) toxicity tests to ensure that freshwater mussels are protected from injury from toxic metals in TSMD streams. In this study, we conducted water-only tests to evaluate the contributions of metals from stream water to toxic effects on juvenile mussels. A 2016 survey of TSMD streams found that dissolved zinc concentrations in surface water and interstitial water of mussel habitats frequently exceeded 10 ug/L at most sites downstream of mining activities. Lead and cadmium were detected in fewer samples, with these metals occurring at ratios of about 200 (Zn): 2 (Pb): 1 (Cd). We conducted 12-week tests with juvenile fatmucket *(Lampsilis siliquoidea*) to compare toxic effects of zinc exposure with the effects of a zinc-lead-cadmium mixture. Results of these studies indicated that toxic effects on juvenile mussels (reduced biomass) occurred at lower Zn concentrations in the metal-mixture test, with an EC20 of 7.2 ug/L (expressed as Zn) in the three-metal mixture, compared to 21 ug Zn/L for the Zn-only test. These results demonstrate that juvenile fatmuckets are highly sensitive to toxic effects of zinc and other metals at concentrations occurring in mussel habitats of TSMD streams. Ongoing studies in our laboratory will characterize the relative contribution of metals from TSMD sediment and overlying water to toxic effects on juvenile mussels, with the goal of establishing a reliable site-specific threshold for injury to mussel communities of the TSMD caused by metals.

## Impacts of Unconventional Gas Operations on Surface Waters, Soils and Groundwater – An International Perspective

**RP101 Development of conceptual models to screen deep groundwater contaminant pathways associated with coal seam gas chemicals**

*D. Mallants, CSIRO Land and Water; J. Kear, Z. Chen, CSIRO; S. Apte, Centre for Environmental Contaminants Research*

The focus of this paper is on developing and evaluating conceptual models with plausible fate and transport release pathways associated with coal seam gas (coal bed methane) development in Australia. The conceptual models developed throughout this study include the following elements: description of the contaminant source(s) relevant to deeper groundwater, including below-ground chemical leaks within the target coal formation and leaks in beneficial aquifers for several types of chemical, the likely fate and transport pathway of the chemical. hydrogeological conceptualisations of typical Australians CSG basins. description of the receptors (groundwater use and groundwater dependent ecosystems, including wetlands, springs, streams). statement of underpinning assumptions and hypotheses. Building on a literature review on plausible fate pathways, four plausible fate and transport release scenarios have been developed for which the likelihood for each of these scenarios is defined. The following scenarios are considered to represent a sufficiently broad range of flow conditions that may provide pathways for chemical transport into aquifers: Fracture growth into an overlying aquifer: This scenario considers hydraulic fracture fluid loss into an overlying aquifer. The scenario considers site conditions that favour height growth of a vertical hydraulic fracture upward towards and into a shallower aquifer. Fracture growth into a well through pre-fracturing permeability and new fractures: This involves two wells within the same coal seam, connected by a pre-existing hydraulic facture; Well rupture during injection: This scenario considers rupture of a cased well during a fracturing injection operation. If a cased part of the wellbore that passed through an aquifer were to rupture while a fracturing fluid was being pumped down the casing, the fracturing fluid could escape directly into the aquifer. Fracture growth into a fault: assessment of leakage potential via a fault that connects the coal seam to an overlying aquifer. For each of the above plausible failure scenarios that may lead to contamination of groundwater resources following hydraulic fracturing operations, the study determines i) if these failure scenarios are physically possible, and, ii) if so, under what properties and conditions, as well as iii) the envelope of possible behaviour.

**RP102 Chemical behaviour of fracturing fluid additives under high pressure and temperature conditions**

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Environmental risk assessment relies on conceptual box models to predict chemical behaviour in the environment. These models are generally based on surface exposure routes and atmospheric physico-chemical parameters. However, during hydraulic fracturing, chemicals may be released deep underground, where high pressure and temperature conditions exist. One previous study on the downhole transformation of glutaraldehyde found that temperature, pH and salinity had the greatest effect on environmental fate. This study aims to broaden this information by assessing the environmental fate of a mixture of three fracturing additives under downhole conditions. 1,2-benzisothiazolin-3-one, phthalic acid and adipic acid were prepared together with crushed shale in 10mL Teflon tubes in an anoxic environment. These samples were then left under different temperature (25?C and 100?C) and pressure (1bar and 450bar) conditions for three days in order to compare degradation, transformation, dissolution and sorption under atmospheric conditions to those found under downhole conditions. Chemical sorption and transformation was assessed by accelerated solvent extraction followed by high resolution mass spectrometry. Dissolution and transformation in the liquid phase was determined by high resolution mass spectrometry. The hypothesis is that the chemical processes will be accelerated by the rise in temperatures. Pressure is not expected to affect these processes significantly. If downhole conditions have no to very little effect on chemical fate then the current conceptual box models may be used to adequately assess chemical exposure related to unconventional hydrocarbon activities. However, if downhole conditions have a significant effect on chemical fate then the models need to be revised for this purpose.

**RP103 Gas Chromatographic-Mass Spectrometric (GC-MS) Determination of Pyridine Bases in Environmental Samples**

*V.O. Olaoluwa, University of Hull / Chemical Engineering*

*Pyridine*, a volatile to semi-volatile organic material, is determined by Gas Chromatography (GC) using Mass Spectrometric (MS) detection in environmental samples such as river water and bottom sediment. A measured sample is collected in a beaker, and is preserved cryogenically (low temperature) with ice usually at $4^0$C to help prevent pyridine from becoming volatilised. The ensuing stage is the extraction process carried out by dissolving the sample in an organic solvent mixture of *n-Hexane* and *Dichloromethane* (DCM) in order to affirm absolute purification process. The pyridine bases are concentrated on a rotary evaporator and determined by Capillary Gas Chromatography-Selected-Ion-Monitoring (GC-SIM). The determination of a number of pyridine bases is done using a 15mm × 0.53mm Ion Detection (ID) Capillary Column followed by Selected-Ion-Monitoring (SIM). The detection limits were 10-15µg/L and percent recoveries from river water and bottom sediment were over 84%. This method is capable of simultaneous determination of sixteen pyridine bases with sufficient sensitivity and accuracy to be applicable to environmental samples.

**RP104 Heavy Metal Concentrations in Surface Water and Sediment from Soku Oil Field, Niger Delta, Nigeria**

*H. Omogoriola, Nigerian Institute for Oceanography and Marine Research / Fishery Resources; S.O. Ayoola, Univeristy of Lagos / Department of Marine Science*

Oil industry activities such as exploration, transportation, storage, use and disposal, as well as oil spills are sources of major contamination problems in Niger Delta, which have significant deleterious effects on aquatic organisms. The objective of this study was to evaluate the impact of crude oil spillage and production activities in Soku oil fields. The sampling stations are Soku Gas Plant (SGP), Soku Flow Station (SFS), Soku Gas Plant Town (SGPT) and Abonemma town (ABT) which is reference station. mfor Cadmium, Lead, Nickel and Vanadium respectively. The heavy metal concentrations in sediment samples also ranged between 0.018mg/l (WT) and1.676 mg/l (SFS), 1.057 mg/l (WT) and 3.985mg/l (SFS), 0.040mg/l (WT) and 0.611mg/l (SFS), 0.004mg/l (WT) and 0.210mg/l (SFS) for Cadmium, Lead, Nickel and Vanadium respectively. The mean concentrations of Cd, Pb, Ni and V in Soku Oil Field are 0.018mg/l, 0.054mg/l, 0.008mg/l and 0.003mg/l for surface water and 0.849mg/l, 2.815mg/l, 0.310mg/l and 0.124mg/l respectively. The decreasing trend of metals in surface water and sediment were Cd>Pb>Ni>V and Pb>Cd>Ni>V respectively. These results showed that heavy metals concentrations in sediments were high than that of water. Also, heavy metals concentrations in water and sediment were above the permissible limit by FEPA/DPR, USEPA and WHO. The areas sampled had high heavy metals concentrations that are likely to crude oil spillage and production activities. It could be concluded that the Soku Oil Field is heavily polluted which could have negative impacts on aquatic organisms inhabit the area.

## Water and Land Management to Reduce Methylmercury Bioaccumulation

**RP105 Trends in fish mercury concentrations within a newly restored tidal marsh at Hamilton Airfield**

*M. Herzog, J.T. Ackerman, A. Hartman, M. Toney, USGS / Western Ecological Research Center*

The Hamilton Army Airfield, in San Pablo Bay, California, was recently restored to tidal action in 2014, and methyl mercury production and bioaccumulation was identified as a potential impact of the tidal marsh restoration. From 2013-2016, we analyzed 331 stickleback for total mercury concentrations from at least 9 sites each year: 2 sites within Hamilton's tidal wetland area, 2 sites within Hamilton's western seasonal wetland, 3 sites within Hamilton's southern seasonal wetland, and 2 reference sites at the Sonoma Baylands. Total mercury concentrations in stickleback were higher at Hamilton than at the Sonoma Baylands reference site in 2013, marginally higher in 2014, and not significantly different in 2015. However, in 2016 stickleback mercury concentrations at Hamilton were once again higher than at the control site. In 2014, stickleback mercury concentrations increased at Hamilton (23% increase) and the Sonoma Baylands reference site (63% increase) relative to 2013 (pre-breach). In 2015, stickleback mercury concentrations showed little change at Hamilton (-1%) relative to 2014, but concentrations at Sonoma Baylands continued to increase during the same timeframe. In 2016, stickleback mercury concentrations increased by 39% at Hamilton, however there was no change in mercury concentrations within stickleback collected at Sonoma Baylands. At Hamilton, stickleback mercury concentrations trends differed depending on the habitat type, increasing by 24% at the tidal wetland, increasing 123% at the southern seasonal wetland, and decreasing by 49% at the western seasonal wetland, between the pre-breach sampling period (2013) and the first post-breach sampling event (2014). These results suggest both localized changes at the Hamilton restoration site, likely related to the breaching event, as well as regional inter-annual variability in fish total mercury concentrations. In general, stickleback mercury concentrations at Hamilton (0.46 µg/g dw; N=331 fish, 2013–2016) were considered high in mercury contamination and comparable to the high concentrations of mercury we have observed in stickleback collected in other mercury contaminated managed ponds, mudflats, and sloughs in the South San Francisco Bay (means range from 0.41-0.45 µg/g dw).

**RP106 Mercury contamination in birds helps guide wetland management decisions for the South Bay Salt Pond Restoration Project**

*J.T. Ackerman, M. Herzog, A. Hartman, USGS / Western Ecological Research Center*

Mercury contamination is elevated within the San Francisco Bay, California and the estuary is a hotspot for mercury contamination of birds within western North America. The South Bay Salt Pond Restoration Project in San Francisco Bay is the largest tidal wetland restoration project on the West Coast and is in process of converting >15,000 acres of former salt ponds into tidal marsh and managed wetland habitats. This large-scale wetland restoration may change the distribution, bioavailability, and bioaccumulation of methylmercury within the estuary and therefore bird mercury concentrations are being monitored to evaluate the effects of management actions. We documented a rapid and substantial response of mercury contamination in Forster's tern eggs following wetland restoration actions. Specifically, egg mercury concentrations increased by 63% (+0.90 µg/g fww) the year after wetland restoration actions, but were similar between years at reference wetlands (-9% or -0.12 µg/g fww). The increase in Forster's tern egg mercury concentrations to a mean of 2.34 µg/g fww in restored wetlands were several times higher than common toxicity benchmarks. The increased mercury concentrations in Forster's tern eggs occurred in the year immediately following the restoration actions, but egg mercury concentrations thereafter declined to ambient levels within 2 years. In contrast, the change in American avocet egg mercury concentrations immediately after wetland restoration actions (-1% or -0.00 µg/g fww), relative to reference wetlands (+1% or +0.00 µg/g fww), was negligible. Bird egg mercury concentrations in restored wetlands remain among the highest in San Francisco Bay and long-term trends in egg mercury concentrations remain elevated. Mercury contamination in birds may be acting as an additional stressor at a time when breeding bird populations are declining in San Francisco Bay and managers and stakeholders are attempting to reverse these population declines by constructing additional nesting islands within wetlands.

**RP107 Sediment Incubations to Assess Mercury Methylation Dynamics in a Hyper-eutrophic Reservoir**

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Inorganic mercury (Hg), predominantly from widespread atmospheric deposition, but also from point sources including mine and industrial sites, is transformed to methylmercury (MeHg) by anaerobic bacteria in oxygen-poor water and sediment. Regulatory measures aimed at reducing MeHg in fish tissue were recently enacted in California and 131 reservoirs are currently identified as Hg impaired. While there is regulatory pressure on reservoir managers to lower fish tissue MeHg concentrations, food web dynamics are complex and there is uncertainty as to how to accomplish this goal. This study focuses on a Hg impaired, hyper-eutrophic reservoir in San Diego, CA where thermal stratification results in the accumulation of dissolved MeHg in the reservoir hypolimnion from April to October. This presentation will present the results of laboratory incubation experiments conducted monthly with reservoir sediment and bottom water. The experiments are designed to asses which conditions are favorable for Hg-methylation, which conditions suppress methylation, and how this changes with time. Incubation treatments include the use of selective bacterial inhibitors, organic substrate amendments, inorganic mercury, and redox manipulation to assess the key drivers and microbial groups involved in MeHg production in this system. Preliminary results suggest that a variety of anaerobic organisms are involved in both methylation and demethylation throughout stratification and that the system is carbon limited soon after stratification begins, but this limitation is quickly met after surface eutrophication results in organic matter deposition to the sediment. These results will be discussed in association with potential approaches to repress MeHg accumulation in fish tissue. With a more comprehensive understanding of MeHg cycling at the profundal sediment-water interface, reservoir managers will be better able to develop effective management strategies aimed at repressing MeHg bioaccumulations in lakes and reservoirs.

**RP108 Seasonal methylmercury dynamics in and export from the Hells Canyon Reservoir Complex, Idaho and Oregon, USA**

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Anoxia in the hypolimnion of lakes and reservoirs can promote the conversion of mercury (Hg) to the more toxic methylmercury (MeHg) form. In the 200-km Hells Canyon Reach of the Snake River along the Idaho-Oregon border, three deep (up to 90 m) reservoirs seasonally stratify for months at a time, creating anoxic conditions that promote MeHg production in the hypolimnion. As a result, both Idaho and Oregon have listed this reach of the Snake River as impaired for Hg, with fish-tissue samples regularly exceeding Idaho's human health fish tissue criterion of 0.3 mg/kg wet weight. In 2014, the U. S. Geological Survey and Idaho Power Company initiated a collaborative investigation of Hg cycling and fate in the Hells Canyon reach. Primary research questions for this project include understanding the mechanisms promoting MeHg production in the hypolimnion, and the fate of the MeHg accumulated in the hypolimnion subsequent to reservoir destratification and mixing. To help answer these questions, the mass loadings of Hg and MeHg into, within, and out of the Hells Canyon complex of reservoirs were estimated using discrete water-quality data and streamflow into and out of each reservoir. Water samples were collected biweekly from four reservoir inflow/outflow locations between 2014 and 2017 and were analyzed for dissolved and particulate Hg and MeHg. Streamflow data collected continuously at the inflow and outflow of each of the three reservoirs were used to compute mean daily streamflow values during the sampling period. Using the R software package rloadest, regression models were developed to relate instantaneous Hg and MeHg loads to daily streamflow and seasonal variables. Loads were computed at various time steps over the sampling period to estimate the mass balance for each reservoir in the Hells Canyon Complex and for the complex as a whole. Results indicate that Hg inflow is ~2.9x greater than outflow, and MeHg inflow is ~1.6x greater than outflow, despite clear seasonal production and export of dissolved MeHg from the reservoir complex. Thus, overall, the reservoirs act as Hg and MeHg sinks. Findings from this study are intended to help manage the Hells Canyon Complex to minimize downstream export of Hg and MeHg.

**RP109 Mechanistic Simulations of Total and Methyl Mercury in the Yolo Bypass, a Mercury-Contaminated Floodplain**

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The Sacramento-San Joaquin Delta in California is impacted by legacy mercury contamination from upstream historical mining. Microbial activity in downstream aquatic environments can convert inorganic mercury to methylmercury (MeHg), a toxic and readily bioaccumulated form of mercury. To protect human and wildlife health, the Delta Mercury Control Program was adopted in 2011. One the requirements of the Program is that MeHg loads from an area known as the Yolo Bypass be reduced from 100 g $yr^{-1}$ to 22 g $yr^{-1}$, a 78% reduction. The Yolo Bypass is a 24,000 ha floodplain used for flood control for the Sacramento River. The Yolo Bypass is also used for a variety of farming and wetland management purposes, and experiences seasonal wetting and drying cycles. The California Department of Water Resources (DWR) is required to evaluate whether operational changes or other management strategies could be implemented to reduce open water MeHg production. A mechanistic model of mercury cycling (D-MCM) is being applied in 2D mode to the Yolo Bypass to identify options to meet regulatory requirements. A 47 cell model grid was used to represent the Bypass in simulations from 1997-2012. Different land uses were assigned to various areas. Hydrodynamics were provided by TUFLOW, a 1D/2D hydrodynamic model. Soil/sediment resuspension rates were estimated by combining hydrodynamic predictions with USGS erosion microcosm experiments. Water column and sediment/soil concentrations of total mercury and MeHg are being measured by DWR and the Moss Landing Marine Laboratories (MLML). Pore water mercury fluxes and the effects of vegetation senescence on MeHg production are also being studied experimentally by MLML to provide information for modeling. Results of the model calibration to existing conditions will be presented, including major sources, sinks, and concentrations of total mercury and MeHg in the Yolo Bypass. Results from the Yolo Bypass simulations are also being used in a broader effort to simulate mercury in the overall Delta.

**RP111 Long-range atmospheric deposition of toxic heavy metals within Kejimkujik National Park, Nova Scotia**

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Kejimkujik National Park, in Nova Scotia, Canada, is a sensitive region for heavy metal contamination, such as mercury, in part due to long-range atmospheric deposition from global and regional industrial regions. The region is remote from industrial centres, but is downwind of major pollution sources in North America and Canada, and historically had numerous gold mining sites. The region has also experienced anthropogenic acidification from sulphate deposition over the 20th century, which has resulted in limnological conditions favourable for mercury (Hg)

methylation within Kejimkujik lakes. Kejimkujik is therefore known to be a hotspot for methylmercury (MeHg) bioaccumulation and biomagnification, with the highest Hg concentrations detected within common loon (*Gavia immer*) populations across Canada and North America. Due to a paucity of long-term atmospheric deposition monitoring in this region, little is known about the response of Kejimkujik lakes to multiple changing global, regional, and local atmospheric Hg and metals sources. Here we use multiple lake sediment cores to reconstruct anthropogenic depositional fluxes of 45 elements, including Hg and heavy metals of concern for the last ~150 years. Results show that Kejimkujik lake sediments are highly enriched in Tin (Sn), Antimony (Sb), Lead (Pb), Mercury (Hg), Bismuth (Bi), Silver (Ag) and Tungsten (W), with current metal concentrations being > 2-fold greater than natural baseline/pre-industrial levels (prior to ~1800 AD). Modern depositional Hg fluxes matched well with precipitation data for the region, with wet deposition measurements and sediment derived fluxes ranging between 5.5 to 7.8 $\mu g/m^2$ and 8.2 to 9.3 $\mu g/m^2$ respectively, between 1990 AD and 2010 AD. Furthermore, lake sites closest to historic gold mining sites show spikes in Ag, Cadmium (Cd), Sb, Thallium (Tl), Zinc (Zn) and, W during the operative period of the mines (~ 1880 AD and 1950 AD). Landscape scale fluxes will also be calculated, and the influence of the catchment area on metal deposition within these Kejimkujik lakes will be explored, along with acid mobility of certain metals within the sediment record. Sediment-core reconstructed atmospheric deposition values combined with biological monitoring data from sensitive regions, such as Kejimkujik National Park, will be useful for predicting the impacts of the Minamata Convention on Hg deposition to lakes and Hg levels in biota.

## Modeling Oil and Chemical Transport in the Water Column After Spills

**RP112 Modeling Water Column Exposure Dynamics from Oil Spills: Model Validation for the Deepwater Horizon Oil Spill**

*D.P. French-McCay, M. Horn, D. Crowley, D. Mendelsohn, RPS Ocean Science*

Oil spill modeling of the 2010 Deepwater Horizon discharge was performed to evaluate the fate and concentrations of the oil and its components in subsurface and surface waters of the Gulf of Mexico, including consideration of the time-varying applications of dispersants at the discharge location at depth and on the surfaced oil. The oil fate model Spill Impact Model Application Package (SIMAP) was used to estimate rise rate of oil droplets released at depth, oil surfacing, weathering (evaporation, dissolution of oil constituents, degradation, emulsification) of oil droplets and surfaced oil, entrainment of surface oil, movements of oil droplets and dissolved components, adherence of oil droplets to suspended particulate matter (SPM), adsorption of semi-soluble hydrocarbons to SPM, sedimentation, and stranding on shorelines. As sampling in deep water (>40 m) during April-July 2010 was primarily performed within 25 km of the spill site, model predictions below 40 m were compared to field data from within a 25 km by 25 km box centered on the wellhead. Concentrations of soluble and semi-soluble hydrocarbon components predicted by the model agreed well with chemical measurements when compared as frequency distributions within varying depth zones of the water column. The results showed that the soluble hydrocarbons primarily dissolved near the release depth, while semi-soluble compounds (e.g., PAHs) were partially dissolved at depth and as droplets rose. Because the sampling in the upper 40 m was not synoptic or sufficiently representative of the entire modeled domain, a comparison of the modeled to observed concentrations was performed using samples collected within the upper 10 m of the 25 km by 25 km box centered on the wellhead between April 22 and July 15 of 2010. Concentration measurements below 0.1 ug/L total PAHs were considered biased due to many constituent concentrations being below method detection limits. The model predictions were that about 70% of the water samples would have concentrations >0.1 ug/L total PAHs, whereas about 80% of the available samples in upper 10 m of the comparison area exceeded this threshold.

**RP113 Simulating Chemical Dispersant Application to a Deep Sea Blowout**

*V. Kolluru, M.J. Fichera, ERM, Inc.*

During the Deepwater Horizon event, chemical dispersants were injected into the flow of oil from the reservoir released into the ocean at the seafloor from the broken blowout preventer through the fallen riser pipe. The objective of the dispersant application was to reduce the droplet sizes of the liquid oil before reaching the water surface. Decreasing droplet sizes increases the surface area of the droplets and thereby increases the rate of weathering and limits the amount of oil reaching the surface that can lead to near-surface biological injury and shoreline impacts. Modifications were made to the Chemical / Oil Spill Impact Module (COSIM) of the Generalized Environmental Modeling System for Surfacewaters (GEMSS®) to extend the existing dispersant application module to simulate the application of subsurface dispersants and its effect on droplet sizes. The surface dispersant module in COSIM was developed using laboratory and field-tested droplet distribution algorithms that depend on pipe diameter of the release, oil properties, blowout jet velocity, Weber number (ratio of inertial to surface tension force), dispersant effectiveness and the droplet distribution curve. In this investigation, the chemical dispersant module was tested on hypothetical blowouts to demonstrate the impact of dispersants on the weathering and the overall mass balance of the simulated oil spill. Modeling results were compared between scenarios made with and without chemical dispersants. Ranges of initial droplet sizes were studied to track the pathways and speed of the rising oil plume while oil dissolves, degrades, and forms a slick on the surface. Variations were also tested for the effectiveness of the dispersants with varying depths of the blowout, altering the time for oil to rise through the water column before formation of a slick.

**RP115 Use of Hydrocarbon Blocks for modeling the transport, fate and exposure of complex mixtures such as crude oils**

*J. Kubitz, Cardno; M.J. Fichera, ERM, Inc.; Y.B. Atalay, S.M. Bartell, Cardno Entrix*

Petroleum products are complex mixtures of many compounds. It is impractical to resolve, quantify and model each individual constituent in a crude oil or even a refined product that has a more consistent composition. It is quite practical, however, to identify specific groups of compounds that have similar physical and chemical characteristics and model those. These groups of compounds have been referred to as “cuts” in the past. As analytical techniques for characterizing petroleum mixtures have become more detailed, it has become more practical to characterize oils with a greater number of groups, which are commonly called “hydrocarbon blocks”. In this presentation we will review a method for organizing the information from readily-available chemical analyses for a petroleum mixture into a suite of approximately 20 hydrocarbon blocks that can be readily modeled with the Chemical Oil Spill Impact Module (COSIM) and other oil spill models. We will also discuss how these hydrocarbon blocks can be used to compare modeled data with measured data from field samples, to facilitate field verification.

**RP116 Modeling the photo-enhanced toxicity of organic compounds at the sea surface requires a dual dose approach**

*J. Kubitz, Cardno; Y.B. Atalay, S.M. Bartell, Cardno Entrix*

The toxicity of some polycyclic aromatic hydrocarbons (PAHs) has been observed to increase markedly when organisms are exposed to ultraviolet (UV) light, which is commonly referred to as photo-enhanced toxicity. There is also evidence that a component of sunscreen formulations may also exhibit photo-enhanced toxicity, and could be adversely affecting coral reefs. The severity of adverse effects depends on both the exposure to individual PAHs and the UV irradiance, which requires both doses to be characterized. In this presentation we will outline the conceptual model of the processes that are involved in photo-enhanced toxicity and describe the computational algorithms that are available for quantifying exposures. We will review the strengths and weaknesses with each algorithm, assess the uncertainties associated with the current state of the science, and recommend areas for future research and development in the quantification of photo-enhanced toxicity.

**RP117 Adsorption of crude oil on activated carbon**

*D.O. Adesina, University of Lagos, Department of Chemistry*

The presence of dissolved crude oil in water poses significant environmental hazards to aquatic lives. Components of dissolved oil, BTEX which are carcinogenic can cause cancer after a long time of exposure. Activated carbon was used to remove both dissolved and dispersed oil in simulated produced water. It was conditioned to provide good oil uptake in its natural form. Investigations carried out include the effects of parametric variations of different adsorbate and time, on the adsorption of crude oil. Sorption experiments with the activated sorbent showed that Temkin isotherm ($R^2$= 0.9548) fitted better than Fruendlich and Langmuir isotherm. Several kinetic models were tested and it was discovered that the sorbent followed pseudo-second order sorption kinetics. The value of Qe deduced from the slope of the curve was 1.01 mg/g and the value of rate constant ($k_2$) was found to be 3.35 $g.mg^{-1}min^{-1}$. This result showed that activated carbon is a good sorbent for crude oil removal in produced water. It will provide a way of cleaning oily contaminated water environment thus safeguarding human health, aquatic lives, and soil fertility.

**RP118 Parting the Tides: Determining Oil Concentration on the Hudson River's Surface Through Liquid-Liquid Extraction**

*C. Renaud, Marist College / Department of Environmental Science; Z. Gagnon, Marist College / School of Science*

The Hudson River is a vital region that not only is home to many essential aquatic ecosystems but is also a water source for public communities in the surrounding area. Protecting this resource is of utmost importance. However, the U.S. Coastguard's recent Hudson River Anchorages Proposal aims to establish 43 new anchorages at 10 sites along the Hudson River. These anchorage sites would heavily increase barge traffic and crude oil transport along the River which is a major concern because these barges, by design and by defect, leak crude oils into the Hudson River. Prior research on water quality safety standards suggest that oil and grease concentrations as low as 0.1 mg/L can be lethal to some of the more receptive aquatic organisms. For drinking water, the Sri Lankan Standards Institute recommends that oil contamination not exceed 0.2 mg/L. Assessment of oil contamination is one of the first steps in determining the severity of the issue. Through this research, an efficient and cost-effective method was developed to determine oil concentration on the surface of the Hudson River. Samples were collected from the River surface in half mile increments away from the Poughkeepsie Waste Water Treatment Plant. The method in question involves using the solvent n-hexanes in liquid-liquid extraction of the oil contaminants, and then comparing the weight of 10 mL n-hexanes solutions before and after extraction. In the preliminary trials of this method, it was determined that the oil concentration on the Hudson River surface exceeded the maximum health safety standards for aquatic life and for public consumption, with the average oil concentration being 1.324 mg/L. This research will aid in the communication of the dire situation that the Hudson River is already facing in order to demonstrate that the River's health cannot afford more barge traffic.

## Adverse Effects of Chemicals on the Microbiome

**RP119 Understanding how dietary exposure to single-walled carbon nanotubes may alter the composition and functionality of the gut microbiome**

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The efficiency of the gastrointestinal system is heavily influenced by the community of microbes lining the gastrointestinal tract (aka "gut microbiome"), which plays a role in metabolism, nutrient absorption, and immune function. Despite the essential role of the gut microbiome in maintaining gastrointestinal homeostasis, interactions between toxic chemicals and the microbiome are not well understood. Carbon-based nanomaterials, especially single-walled carbon nanotubes (SWCNTs), have emerged as a class of contaminants of particular concern due to their high production volume and increased likelihood of environmental release. In aquatic environments, these hydrophobic nanomaterials likely settle onto sediments and associate with organic matter, where they may enter the food chain through dietary routes. Recent evidence from our group has found that, while SWCNTs themselves are not physically taken up by the gastrointestinal system, they can induce molecular changes at the gastrointestinal interface, altering the regulation of genes encoding for the peptide transporters, *pept1* and *pept2,* and the satiety hormone cholecystokinin. These findings indicate that SWCNTs can interact with the gastrointestinal environment; however, the specific interactions and their consequences remain unclear. In order to investigate how dietary exposure to SWCNTs may change the gastrointestinal environment, we conducted a 3-month feeding study using largemouth bass (*Micropterus salmoides*), a top level freshwater predator likely to be exposed to SWCNTs through dietary routes. Fish were exposed to 2.5 mg/kg SWCNT-coated food and fed once daily to satiation. At 0, 4, and 11 weeks of exposure, guts were removed, sectioned into proximal, middle, and distal parts, and stored at -80 for analysis. Next-generation sequencing followed by PiCrust modeling was used to identify differences in the composition and functionality of the gut microbiome associated with dietary exposure to SWCNTs. Additionally, a combination of analytical, and molecular methods where used to observe SWCNT-induced changes in endpoints such as gut inflammation and bile interactions. Data from this study will increase our understanding of impacts of chemicals on the gastrointestinal environment and help elucidate potential environmental risks associated with exposure to single-walled carbon nanotubes.

**RP120 Effects of oil on gut and gill microbiome of three pelagic Gulf of Mexico fish**

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The microbiome of fish is a crucial part of maintaining host health, and helps with acquiring nutrients in digestion, developing the immune system, and protecting the host from opportunistic pathogens and other environmental stressors. Microbial communities can vary in species composition and have functionally different roles depending on their location within the host, including the gastrointestinal tract, skin, and gills. However, little is known about the microbial community of marine fish and their response to environmental stressors. The Deepwater Horizon disaster was the second largest oil spill in history, and had catastrophic effects on several commercially and ecologically important fish species in the Gulf of Mexico. This study aims to determine if exposure to oil will affect gut and and gill-associated microbiomes similarly in different species. We will accomplish this by sequencing the microbiomes of three pelagic species (Red Drum, Atlantic Croaker, and Red Snapper) exposed to oil along with a control group. This will allow us to gain insight on the core microbiomes of these species, compare the differences between pelagic and benthic species, and determine how microbial communities respond after exposure to oil.

**RP121 The effect of the plasticizer (Di-2-(ethylhexyl) phthalate) on microbiome-gut axis in zebrafish**

*O. Adamovsky, Masaryk University / Department of Physiological Sciences; A. Buerger, J.H. Bisesi, University of Florida / Environmental and Global Health; C.J. Martyniuk, University of Florida / Physiological Sciences*

Di-2-(ethylhexyl) phthalate (DEHP) is a plastic softener (plasticizer), that is currently used in products such as PVC, sealants, adhesives, paints, lacquers, printing inks and ceramics. DEHP is found in relatively high concentration in indoor air, surface waters, sediments, and perhaps most alarmingly, in municipal drinking water. DEHP is an emerging environmental contaminant of concern for human health due to ubiquitous and widespread use and for their potential to disrupt metabolic processes. Recent studies suggest that microbiome and gut play significant role in metabolism and disruption of microbiome-gut axis leads to effects on intake of nutrient, hormone balance, immunity and nervous system. The goal of this study is to determine whether DEHP, a model metabolic disruptor, adversely affect the zebrafish (*Danio rerio*) microbiome-gut axis and describe how taxonomical change in microbiome are reflected in gut. To stimulate chronic exposure of DEHP, we exposed the fish to 3 ppm DEHP via food for 30 and 60 days. Fish were fed once a day with commercial food mixed with DEHP. Fish were dissected and the content of gut and the gut tissue were collected. The composite and diversity of microbiome were studied by analyzing of 16s RNA genes using Illumina MiSeq. Sequence data were then separated into operational taxonomic units (OTUs) and annotated via mapping to a bacterial genome. OTU table was then used to link the phylogeny and the function of the microbiome using a bioinformatics approach PICRUSt. To identify genomic responses of DEHP in gut, we used microarray and real-time RT-PCR analysis followed by differential gene expression analysis. The data were then mapped to changes in cell processes and signaling pathways using Pathway Studio. This study revealed, that DEHP can significantly effects the composition of microbiome and its related function, and that these microbial changes together with the direct effect on gut has an effect on gut's transcriptome. Interestingly, the changes on gut and microbome are different for both sexes. The aim of this presentation is to present the microbiome-gut axis as a target for metabolic disruptors, such as DEHP and emphasize the need of multi-omics approach to study the phenomenon.

## Non-Conventional Exposure Routes and Transport Media of Consumer Product Chemicals to Improve Environmental Policy

**RP122 Leaching of Melamine from Resin Cups at Different Temperatures**

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Melamine-based resins are used in many dishware because of durability, heat resistance and strength. Nevertheless, studies have shown that melamine exposure cause health risks in humans and pet animals. In this study, we evaluated leaching of melamine from dishes under normal cooking conditions. Prior to that a method was developed to extract melamine from water. Graphite carbon column, with π-π interaction sites, was able to extract melamine from water efficiently. Then we leached melamine from 3 types of dishes. The cups were added with 100 mL of water (20?) or hot water (90-100?) and sonicated for 10 min, and extracted by SPE-cartridge. In 20 ? water, melamine concentrations leached into water were in the range of < LOQ (0.04 ng/cm$^2$) to 0.55 ng/cm$^2$ for the three cups. In hot water, melamine leached at 0.37 to 1.25 ng/cm$^2$. Low-priced cups leached higher amounts of melamine than high-priced ones. Older-cups leached higher amounts of melamine than new-ones. These results suggest that melamine based dishware are source of human exposure to these chemicals.

**RP123 Non-Target HRMS for Tracking Sources of Human Contamination to Stormwater Conveyances**

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Fecal contamination presents a risk to human health, however, our incomplete understanding of the sources and relative contributions of fecal contamination and human-specific pathogens in stormwater runoff currently limits the scope and effectiveness of remedial activities. To complement biological measurements, we have developed an assessment tool using high-resolution mass spectrometry (HRMS) to identify significant sources and their relative contributions to effectively manage fecal contamination in stormwater conveyances from Southern California watersheds. This technique has been previously applied to track contaminant flows by comparing chemical fingerprints in urban receiving waters around Puget Sound (WA, USA). Here we demonstrated the utility of HRMS in source tracking of human fecal contamination by chemical fingerprinting of wastewater from different sources (e.g. POTWs, septics, and other non-point sources) and in receiving waterways to distinguish source-specific contributions. Water samples collected from select sanitary sewers and receiving waters were processed by solid phase extraction (SPE) without pre-filtration and analyzed by LC-QTOF-MS in electrospray ionization mode (ESI+). The HRMS data was blank subtracted, qualitatively compared, and statistically analyzed (e.g. hierarchical clustering analysis and fold change) to develop source-specific fingerprints. These fingerprints can then be manipulated, e.g. via characteristic sample dilution, to estimate source contributions in complex samples.

## Aquatic Toxicology, Ecology and Stress Response – Part 2

**RP124 "From single chemicals to complex mixtures" effect of contaminants of emerging concern on fish across three life stages**

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Contaminants of emerging concern (CEC), including pharmaceuticals, personal care products and industrial agents may impact aquatic life. Previous studies have documented reduced escape performance in fathead minnow larvae exposed to diverse CECs. However, these studies did not consider the complex mixtures of CEC present in the environment. The current study tested the hypothesis that CECs in mixture change fathead minnow development and behavior differently than the mixtures' individual constituent. We assessed the potential of 20 commonly detected CECs to alter both juvenile (escape performance, feeding efficiency) and adult (nest defense, courtship, boldness) behaviors central to survival and reproduction after 96-hour flow-through exposures. We also assessed fathead minnow embryos that are exposed to the same CECs to determine exposure-associated developmental deficiency. In addition, we began the process of building increasingly complex mixtures of CEC using the same compounds. Compound concentrations and mixture composition were based on an analysis of nearly 500 water samples collected as part of the Great Lakes Restoration Initiative. Results to date suggest changes in survival, escape performance and feeding efficiency along with spinal curvature. The survival was significantly reduced ($p< 0.05$, ANOVA) in larvae exposed to estrone, desvenlafaxine, and tris(2-butoxyethyl) phosphate. In both metolachlor and DEET exposures, the medium concentration (environmental concentration) had significantly more non-responsive larvae as part of the escape performance assay as was the case in the highest concentration (10x) of atrazine exposure. Interestingly, several treatments increased the feeding efficiency of larvae under chemical stress. Assessing embryonic, larval, and adult life stages of fathead minnows, exposed to the same CECs, will aid in identifying the most sensitive stage at the molecular, organismal, and apical level of biological organization. We expect that these evaluations will lead us to understand the effect of CECs in mixture on fish survival, development and behavior.

**RP125 Acute microcystin-LR exposure affects the complement C9 synthesis by the interference of thyroid hormones in adult zebrafish (*Danio rerio*)**

*Y. Gao, Yunnan Agricultural University; Z. Liu, D. Li, Huazhong Agricultural University / College of Fisheries*

The persistence of microcystin-LR (MC-LR) in aquatic environments raises potential disturbances in the thyroid hormone (TH) homeostasis, which results in the downstream biological processes associated with TH interference. However, it has been not clarified how MC-LR affect certain downstream biological processes by TH disturbances. In this study, 12 genes were down-regulated in MC-LR treatment zebrafish with at least two-fold change, compared with the control fish through integrative bioinformatics analysis from Gene Expression Omnibus (GEO) datasets GSE12214 and GSE73739. Through Protein-protein Interaction (PPI) analysis between candidates and thyroid genes at the protein level, complement component 9 (C9) was highlighted as one of the most probable molecules among the 19 candidates after exposure to MC-LR. After exposure to 50, 100, 200 and 400 μg/L MC-LR for 24, 48, 72 and 96 hours, some interference effects were observed on the thyroid hormone metabolism of adult zebrafish at thyroxine (T4) and triiodothyronine (T3) levels, iodothyronine deiodinase (Dio) activities and related-genes expression (*crh*, *tsh*, *ttr*, *nis*, *tpo*, *dio*, *thra* and *thrb*) pattern. Acute MC-LR exposure stimulated negative feedback regulation of hypothalamic-pituitary-thyroid (HPT) axis in adult zebrafish with females exhibiting more sensitivity than males. In conclusion, the toxicity of MC-LR may affect the complement system involved in innate immunity through the activated thyroid hormone receptors (TRs) by TH binding to the C9 promotor, regulating membrane attack complex (MAC) function in zebrafish.

**RP126 Analysis of Multiple Cetacean Reproductive and Stress Steroid Hormones in 50 mg Blubber Sample by Liquid Chromatography – Tandem Mass Spectrometry**

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Steroid hormone profiling in cetaceans provides important information for conservation and management. This is critical for endangered species. Until 2017 cetacean blubber hormone analyses were only conducted using immunoassays. This methodology is rarely compatible with multiple analyses in biopsy samples due to mass requirement, and the majority of published immunoassay studies reported using 75-150 mg of blubber per hormone analysis. We report here on a Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) method for the simultaneous quantitation of reproductive and stress steroid hormones in as little as a 50 mg blubber sample. Blubber samples collected by skin biopsy or necropsy and ranging from 50 – 150 mg wet weight (w/w) were extracted for steroid hormone analysis in two cetacean species (beluga *Delphinapterus leucas* and bowhead whale *Balaena mysticetus*). Gel permeation chromatography was employed for lipid removal. Steroid hormones were quantified using a Thermo Scientific TSQ Quantum Access Max MS mass spectrometry system in heated electrospray positive and atmospheric-pressure chemical negative ionization modes through selected reaction monitoring. Isotopically-labeled internal standards were used for endogenous steroids quantitation. Method detection and quantitation limits were compatible with steroid hormone levels reported in cetacean blubber. Method extraction efficiencies were comparable to liquid-liquid extraction. Progesterone, testosterone, cortisol, and estradiol were successfully quantitated from 50 mg w/w cetacean blubber using LC-MS/MS. To our knowledge, this is the first report on pregnancy detection and blubber estrogen analysis by LC-MS/MS. Due to its low mass requirement, this significant advancement in blubber steroids determination makes this method invaluable as it can be accomplished concurrently with other research investigations relying on a single biopsy sample.

**RP127 Antennae Regeneration of the Marine Amphipod *Parhyale Hawaiensis***

*O. Diehl, P. Assano, School of Technology / UNICAMP / LAEG; G.D. Umbuzeiro, School of Technology / UNICAMP / LEAL*

*Parhyale hawaiensis* is a marine amphipod of worldwide circumtropical distribution and has been used in acute ecotoxicological tests. *P. hawaiensis* is able to regenerate its appendages, limbs and tissues after an injury or lost during the entire course of their life. Regeneration can be used as an ecotoxicological endpoint to assess potential teratogenic compounds and their impact on stem cells.. Thus, the aim of this study was to obtain data on regeneration of antennae of *P. hawaiensis* to determine the regeneration time of fifty per cent of population. On day one left antennae of six months old organisms were amputed with sterilized tweezers, each organism transferred to recipients containing 100 mL salt water. Each test consisted of 20 organisms, 10 males and 10 females. During this period, organisms were feed three times a week, the necessary conditions of salinity, temperature, aeration, substrate and luminosity were provided. Four independent experiments were performed. The organisms were monitored daily until all of them undertook full regeneration. Antennae regeneration occured from 7 to 20 days (n=80) after amputation. Males and females behaved statistically differently, although the difference was not biologically relevant ($T_{50\%}$=14 days for males, $T_{50\%}$=12 days for females and $T_{50\%}$=13 days for both). The regeneration time of fifty per cent of the population seems to be viable endpoint. as an ecotoxicological test.To allow testing of toxicants with low Kow and water solubity usually DMSO is used as solvent. To verify its non effect concentration, organisms (n=20 per treatment) with amputed antennae were exposed individually in plastic vials containing different concentrations of DMSO dissolved in 100 mL salt water under the same conditions described above, DMSO can be used as a solvent for toxicants at a maximum concentration of 0.2%. Next steps will be the exposure of organisms to selected toxicants, as growth inhibitor, to verify their ability of affecting the regeneration process in the developed experimental conditions. *Acknowledgement:* Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq-PVE Process: 400362/2014-7) for funding and PIBIC for undergrad fellowship. Amanda dos Santos e Gabriel Rampazzo Magalhães for technical contribution.

**RP128 Atlantic Cod (*Gadus morhua*) Shrinks in Response to Environmental Pollutants Exposure**

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As do global warming and overfishing, environmental pollutants (e.g., polychlorinated biphenyls (PCBs), pesticides and metals) impact marine ecosystems; in particular, its exposures could pose significant risks to fish growth. However, how fishes respond to the exposure of pollutants is poorly understood. Thus, we estimated the effects of environmental pollutants exposures on the lengths (sizes) of Atlantic cod (*Gadus morhua*), using a time series of more than 4,000 individual cod lengths and environmental contaminants levels of cod liver observed in a variety of marginal seas of the Atlantic Ocean (Baltic Sea, Greater North Sea, Norwegian Sea, Barents Sea, and Icelandic Water) in the 1990s and the 2010s. Using linear mixed-effects models, we accounted for spatial population structure and the nested structure of the observation data. Similar to previous studies, the cod sizes increased with the advance of age, and they decreased by the amount of annual landing. Oceanographic parameters also significantly influenced the cod sizes. The sizes were positively associated with salinity and nitrate concentration; however, they were decreased along with increasing dissolved oxygen, phosphate, silicate concentration, and temperature. More importantly, we found a positive correlation between the cod sizes and hexachlorobenzene (HCB) levels; on the other hand, the sizes were negatively affected by cadmium (Cd), lead (Pb), total hexachlorocyclohexane (HCH) and total PCBs levels. These results suggest that environmental contaminants cause cod shrinking, along with ocean warming and overfishing in the Atlantic Ocean.

**RP129 Bivalve responses to graphene oxide exposures**

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Graphene is a two-dimensional nanomaterial composed of $sp^2$ hybridized carbon atoms. Due to its unique mechanical, thermal, electrical, and optical properties, the use of graphene family nanomaterials (GFNs) in electronics, biomedicine, surface coatings, filtration devices, and composite materials is rapidly growing. It is inevitable that with widespread use, GFNs will make their way to aquatic ecosystems. However, current information on fate and toxicity of GFNs, such as graphene oxide (GO), and their environmental impacts is scarce. Filter-feeding bivalves, such as *Crassostrea virginica* (Eastern oysters), are good models to study the effects of GO exposures on aquatic organisms. The goal of this pilot study is to evaluate effects of in vivo GO exposures on oysters using a static renewal design. Each oyster was placed in a beaker with 1 L of 0.22 µm filtered natural seawater that contained 0, 1, or 10 mg/L GO. Oysters were fed a mixed algal diet and three renewals were performed daily. For every renewal, a GO suspension was prepared in seawater and sonicated before its addition to the beakers. Water samples were analyzed for GO concentration and effective diameter after each renewal. After 72 hours of exposure, oysters were placed in clean seawater for 3 hours prior to harvesting gill and digestive gland tissues. Tissues were analyzed for lipid peroxidation, a marker of oxidative damage, and for activity of glutathione-s-transferase (GST), a detoxification enzyme that also participates in oxidative stress and cell signaling pathways. Additionally, gill tissues were fixed for histopathological analyses. Elevated lipid peroxidation in GO exposed oysters was found. No significant changes in GST activity were observed, but reduced total protein levels were noted in exposed oysters. Loss of mucous cells, hemocytic infiltration, and vacuolation were also observed in gill tissues. Results indicate that short-term GO exposures can induce oxidative stress, gill epithelial inflammation, and adversely affect overall health in oysters. Evaluation of sublethal effects of exposures to an emerging contaminant, such as GO, is critical to understanding the risks associated with increasing commercial usage of nanomaterials. Current long-term analyses of exposure effects are aimed at identification of molecular initiating events and subsequent key events that can contribute to the development of adverse outcome pathways (AOPs) for nanomaterials.

**RP130 Chinese medaka (*Oryzias sinensis*): A fish model linking laboratory and field effects in aquatic toxicology studies in China**

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The unprecedented deterioration of the aquatic environments has been occurred in China during the past decades. Ecosystem degradation can be seen in many rivers, and even worse in some rivers where it is difficult to find the presence of fish. So, toxicity investigations of contaminants in aquatic environments on fishes are of great concern. But there is often embarrassment when linking the effects found in traditional model fishes in the laboratory and the phenomena observed in wild fishes in the field due to species differences. Field caging experiments are sometimes effective, however, experimental animals and their eggs or offspring would accidentally be released into the environment and cause alien invasion. So developing a new fish model from native species for laboratory study should be preferred. Here we select Chinese medaka (Oryzias sinensis), a close relative species of Japanese medaka (Oryzias latipes), and established a laboratory brood stock of Chinese medaka. Using next generation sequencing (NGS) technologies and modern bioinformatics tools, we got the genome and transcriptome of Chinese medaka. And finally, the fish model was successfully used to analyze and identify the key hazards to fish populations in some rivers based on experiments at molecular, cellular and individual levels.

**RP131 Comparative toxicity of a nano-TiO2, a bulk TiO2, and a food additive TiO2 to *C. elegans* – Does the size matter?**

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Bulk-sized $TiO_2$ has been used as food additives for years and is considered safe for human and ecosystem health because of its inertness. Recent studies found that food additive $TiO_2$ contains significant portion of nano-sized (i.e., < 100 nm) $TiO_2$ which may pose unforeseen health risks for humans. Here we investigated toxicity effects of a commercial food additive $TiO_2$ as compared to nano-sized $TiO_2$ (P25) and bulk $TiO_2$ to a model organism the nematode *C. elegans*. Particle size characterization was performed using scanning electron microscopy (SEM) and X-ray diffraction (XRD). Photoreactivity (generation of reactive oxygen species) of the materials were determined using a fluorescence-based assay as well as methylene blue degradation. Toxicity endpoints included acute phototoxicity (lethality), reproduction, and lifespan. Uptake of $TiO_2$ particles was assessed by microscopy imaging followed by hyperspectral imaging. We found that approximately 20% of the food additive $TiO_2$ had particle size below 100 nm. Overall, food additive $TiO_2$ had similar size distribution to bulk $TiO_2$, with average primary particle size of 148 nm (range: 53, 308) and 129 nm (64, 259), respectively. Under environmentally relevant UV radiation, P25 showed significantly higher photoreactivity and phototoxicity than food additive $TiO_2$ and bulk $TiO_2$, with a 24-h LC50 of 8.7 mg/L (95% CI: 8.5, 8.9). All three materials induced concentration-dependent impact on worm reproduction at concentrations up to 10 mg/L, and P25 showed a greater impact at an identical concentration. All three materials impacted worm lifespan and P25 showed the greatest effect, shortening lifespan by 2 days. In addition, worms exposed to all three materials showed age-associated vulval integrity defects during aging, with a frequency of 30~50% as compared to less than 15% in control worms. Uptake of $TiO_2$ into worm intestinal system was obvious for all three $TiO_2$ materials; however, uptake into other organs was most significant for P25, followed by food additive $TiO_2$, and no significant uptake of bulk $TiO_2$ was observed. Comparable toxicity effects between food additive $TiO_2$ and bulk $TiO_2$ correspond well with their similar primary particle size and size distribution, suggesting that particle size is a determining factor for toxicity in this case. Future studies should focus on the uptake of $TiO_2$ particles into different organs of the worm and the associated biological effects from molecular and cellular levels.

**RP132 Comparative Transcriptomic Response of a Model Fish and Amphibian to Trifloxystrobin Early Life Stage Exposures**

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Size at metamorphosis and time to metamorphosis indicate developmental effects in amphibians that could result in population-level impacts. Current risk assessment practice uses early life stage fish data when data for larval amphibians are not available. While fish are sufficiently sensitive to a range of chemicals, developmental delays could have relatively greater impacts on subsequent amphibian life stages. As pesticide exposure has been suggested to contribute to amphibian declines, it is important to understand the uncertainties around using this form of surrogacy in risk assessments aimed at protecting amphibian populations. Strobilurin fungicides are an economically important class of pesticides designed to inhibit the mitochondrial respiratory chain ultimately interfering with ATP production. Trifloxystrobin (TX), a heavily used strobilurin, is frequently detected in aquatic systems, often at levels known to cause sub-lethal toxicity in a range of species. In this study we conducted 10-day early life stage exposures of zebrafish, *Danio rerio*, and African clawed-frog, *Xenopus laevis*, to TX to determine if growth, development, and gene expression changes were similarly altered. Log-logistic 96-hr LC50 curves for *D. rerio* and *X. laevis* show that *X. laevis* is more sensitive to TX exposure than *D. rerio* with LC50 values of 45.85 ± 3.98 µg/L and 135.86 ± 7.58 µg/L, respectively. The LC50 for the entire 10-day exposure period was more similar between *D. rerio* and *X. laevis* at 79.13 ± 4.61 µg/L and 45.61 ± 3.83 µg/L, respectively. While a delay in hatching and pigmentation was observed in *D. rerio,* no effects on length or weight were observed. However, a dose-dependent decrease in weight and developmental delay were observed in *X. laevis* as fewer TX-exposed tadpoles reached NF stage 50 by the experiment termination than control tadpoles. Tissues collected from exposed individuals were used to compare differential gene expression on targets related to endocrine signaling, oxidative stress, mitochondrial function, growth, and apoptosis. Species-specific gene expression patterns suggest that different biological pathways in *D. rerio* and *X. laevis* are perturbed by TX exposure. Future studies will examine the potential of the observed changes in gene expression to be predictive of more population-relevant endpoints, such as time to metamorphosis.

**RP133 Comparing apical responses and molecular toxicity pathways in two amphibian species following chronic exposure to ethinyl estradiol and chlorpyrifos**

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Environmental contaminants have been listed as the second most important threat to amphibians after habitat loss. Exposure to contaminants can have adverse outcomes on amphibian health, such as altered rate of metamorphosis, reproductive effects, immune suppression, and behavioural effects. However, little is known regarding the sensitivity of native amphibian species and even less regarding the specific mechanisms of action and the associated toxicity pathways that drive apical responses to contaminants. Therefore, the main goal of this study is to identify, validate and compare key molecular toxicity pathways that are predictive of contaminant-induced apical responses in the native North American anuran amphibian species, *Lithobates sylvaticus* (wood frog) and the laboratory model species, *Xenopus laevis*. Specifically, this study focused on chlorpyrifos (CPY) and ethinyl estradiol (EE2), two anthropogenic contaminants of concern with different modes of action and characterised apical effects in amphibians. Post-hatch individuals were exposed for 96 h to CPY (0.5, 2, 8 ug/L) and EE2 (0.04, 0.2, 1 ug/L) and sampled for whole transcriptome, whole proteome and targeted metabolome analyses to characterize molecular toxicity pathways following early life stage exposure. A subset of tadpoles was then transferred to a flow-through diluter system for exposure to metamorphosis (~ 40 d) and assessed for developmental stage, morphometrics, organ histopathology and genetic sex. The ultimate goal of this study is to identify critical toxicity pathways for early life stages that enable prediction of key genes to be used in an early life-stage gene expression assay to predict apical outcomes of ecological and regulatory relevance in amphibians. This study is part of the EcoToxChip project (@ecotoxchip).

**RP134 Comparisons of the effects of nanoparticles on the activity and biosynthesis processes of β-galactosidase using a mutant strain *E. coli***

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The effects of nanoparticles (NPs) on the activity and biosynthesis of enzyme (β-galactosidase) were compared in the presence of six metal oxide NPs using mutant strain *E. coli*. Various effects were observed according to the types of NPs. In general, the effects on enzyme activity were greater than on that of biosynthesis, except with $Al_2O_3$ NP. Statistically significant differences were observed between these two biological activity (<em>p </em 0.05). For both cases, ZnO and CuO NPs caused the considerably high inhibition among tested NPs. The ECsub50s/sub for ZnO were 0.19 and 3.68 mg/L on the enzyme activity and biosynthesis, respectively. Slightly different orders of ECsub50s/sub were observed as followings: ZnO > CuO > NiO > $Co_3O_4$ > $TiO_2$, $Al_2O_3$ on enzyme activity and ZnO > CuO >NiO, $Al_2O_3$ > $TiO_2$, $Co_3O_4$ on the biosynthesis process. The causes of this different phenomenon are unclear at this point. More research will be needed to be confirmed with further in-depth studies, such as molecular level investigations, to elucidate the different mechanism of the toxicity.

**RP135 *Daphnia magna* assay application to toxicity of nickel compounds**

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Various inorganic chemicals exist in environment, their come from not only natural source but also artificial source. Inorganic chemicals include metal, metal ion, metal salts, and nano materials. We expect that the metal salts also affect organisms. The following studies mainly use metal salts as inorganic chemicals. Median effective concentrations ($EC_{50}$s) were used from previous studies and several literatures. *Daphnia magna* immobilization test is listed in OECD Guidelines for the Testing of Chemicals (TG202). Culture medium was used Elendt M4. Acute $EC_{50}$s of nickel salts employed values of chloride, nitrate, sulfate, and acetate. Following listed compounds, nickel chloride ($NiCl_2$: $NiCl_2$?$6H_2O$, CAS No. 7791-20-0), nickel nitrate ($Ni(NO_3)_2$: $Ni(NO_3)_2$?$6H_2O$, CAS No. 13478-00-7), nickel sulfate ($NiSO_4$: $NiSO_4$?$6H_2O$, CAS No. 10101-97-0), and nickel acetate ($Ni(CH_3COO)_2$: $Ni(CH_3COO)_2$?$4H_2O$, CAS No. 6018-89-9) were used. Copper sulfate ($CuSO_4$: $CuSO_4$?$5H_2O$ CAS No. 7758-99-8) was used for positive control. Result of acute assay, 48h – $EC_{50s}$ (nominal nickel compound concentration) were 21.6 mg / L ($NiCl_2$?$6H_2O$), 23.5 mg / L ($Ni(NO_3)_2$?$6H_2O$), 5.35 mg / L ($NiSO_4$?$6H_2O$), 11.6 mg / L ($Ni(CH_3COO)_2$?$4H_2O$) respectively. Result of acute assay, 48h – $EC_{50s}$ (nominal nickel ion concentration) were 5.33 mg / L ($NiCl_2$: $Ni^{2+}$), 4.74 mg / L ($Ni(NO_3)_2$: $Ni^{2+}$), 1.19 mg / L ($NiSO_4$: $Ni^{2+}$), 2.74 mg / L ($Ni(CH_3COO)_2$: $Ni^{2+}$) respectively. Result of acute assay, 48h – $EC_{50s}$ (measured nickel ion concentration by ICP-AES) were 0.52 mg / L ($NiCl_2$: $Ni^{2+}$), 0.23 mg / L ($Ni(NO_3)_2$: $Ni^{2+}$), 0.91 mg / L ($NiSO_4$: $Ni^{2+}$), 2.38 mg / L ($Ni(CH_3COO)_2$: $Ni^{2+}$) respectively. These $EC_{50}$s were almost same as previous studies and several literatures. Nickel compounds should be careful each salts, considering measured nickel concentration (e.g. max. 1.6 mg $Ni^2$ / L, 2001 in Japan) from environment water.

**RP136 Developmental hepatotoxicity of PCB-11 in combination with PCB-126 in zebrafish (*Danio rerio*) is dependent upon the window of exposure**

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3,3'-Dichlorobiphenyl (PCB-11) is a non-legacy, lower-chlorinated PCB congener that is an unintended byproduct of diarylide pigment manufacturing and is widely detected in environmental samples. Our previous research in zebrafish (*Danio rerio*) embryos demonstrates that static exposures to PCB-11 alone does not cause toxicity, but in co-exposures starting at 24 hours post fertilization (hpf) with the potent aryl hydrocarbon receptor (Ahr) agonist 3,3',4,4',5-Pentachlorobiphenyl (PCB-126), PCB-11 acts as an Ahr antagonist to rescue the cardiovascular and craniofacial malformations induced by PCB-126. PCB-126 also induces a stunted liver size when exposures begin at 24 hpf. We hypothesized that PCB-11 would rescue this liver phenotype, and to test this, used transgenic *Tg(gut:GFP)* zebrafish to visualize liver development in vivo. Embryo exposures were initiated at different critical windows of liver development: 24 hpf (pharyngula stage, before liver budding), 48 hpf (hatching stage, before liver growth initiation), or 72 hpf (larval stage, during liver extension). Exposure conditions were comprised of static concentrations of either 0.05% DMSO, 20 µM PCB-11, 5 nM PCB-126, or a combination of 20 µM PCB-11 + 5 nM PCB-126. At 96 hpf, a time point when zebrafish livers are extending ventrally, embryos were assessed for gross embryo morphology and liver area. Our results showed that over all critical exposure windows 20 µM PCB-11 alone did not affect gross morphology or liver size of fish. Exposures to PCB-126 starting at 24 hpf were consistent with previous studies, with fish exhibiting severe morphological deformities and a 50% reduction in liver size. Exposures to PCB-126 starting at 48 hpf induced some morphological deformities, but PCB-126 exposures starting at 72 hpf produced normal fish; liver size in fish exposed to PCB-126 starting at 48 hpf and 72 hpf developed normally. In co-exposures starting at 24 hpf, 20 µM PCB-11 rescued stunted liver size and other associated morphological deformities induced by PCB-126. In co-exposures starting at 48 hpf and 72 hpf, fish exposed to 20 µM PCB-11 + 5 nM PCB-126 did not induce gross morphological deformities, however, this co-exposure significantly reduced liver size by 31% and 24%, respectively. This study highlights the importance of examining emerging contaminants in a mixtures context, as well as the usefulness of transgenic zebrafish lines to examine organ-level effects of these mixtures.

**RP137 Difference in zinc tolerance of freshwater snails from contaminated and uncontaminated sites**

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The Tri-State Mining District (TSMD) is a historic mining area located in northeast Oklahoma, southwest Missouri, and southeast Kansas and was an important source of lead and zinc to the United States from the 1850s to the 1970s. These mining operations have resulted in elevated levels of lead, zinc, and cadmium in nearby terrestrial and aquatic environments. The consequences of this contamination include the designation of Tar Creek as an EPA Superfund site in 1983. The present study tested the hypothesis that freshwater snails (*Physa acuta*) found in metal-contaminated waters in the Oklahoma portion of the TSMD will be more tolerant to zinc than those found in less-contaminated waters. Laboratory cultures were developed by obtaining snails from 10 sites across a range of metals contamination within the same watershed. Sublethal zinc toxicity tests (21d) were conducted measuring growth and egg production in response to varying levels of zinc exposure. Preliminary results indicate that snails originally collected in uncontaminated sites are more sensitive to zinc exposure for both growth, reproductive, and mortality endpoints as compared to snails originally obtained from more contaminated sites.

**RP138 Differences between the impacts of acute and sub-chronic NPs exposures on *Gammarus fossarum***

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Silver (AgNPs) and gold nanoparticles (AuNPs) have really specific and unique properties that bring a high interest for their use in different scientific and industrial applications. This increase of their use will ultimately result in their release in aquatic ecosystems and consequently represent a hazard to aquatic organisms. Among those aquatic organisms amphipods are widespread and important component of the aquatic macroinvertebrate assemblage and will certainly be exposed to AgNPs and AuNPs. For these reasons, *Gammarus fossarum* was selected as model organism for this study. The aim of the present work was to evaluate the acute (72h, without food) and the sub-chronic (15d with food) toxicity of AgNPs and AuNPs on *G. fossarum*. In both exposure scenarios, we used $AgNO_3$ as a positive control and as a reference in order to address the contribution the potentially released ions from the AgNPs on the observed effects. The present work evaluated the influence of the surface coating (citrate CIT, polyethylene glycol PEG) on effects induced by the NPs regarding the type of exposure. The uptake of AgNPs and AuNPs by *G. fossarum*, their tissue distribution and the modulation of the expression of stress related genes were investigated in both acute and sub-chronic exposure scenarios. A significant uptake of Ag and Au was observed in exposed animals, with a significant effect of the coating with CIT-NPs showing higher bioaccumulation than PEG-NPs. The analysis of the tissue distribution of the different NPs revealed the presence of the AgNPs in the cuticle and the gills and AuNPs in the gut area in the case of the acute exposure and this profile was kept after the sub-chronic exposure. However, between the acute and sub-chronic exposure conditions two different gene expression profiles were obtained. For instance, for AgNPs, in the acute exposure condition, the *CuZnSOD* expression was impacted without any impact on the expression of *NaKATPase* whereas in the sub-chronic exposure condition a clear impact was visible on the expression of *NaKATPase* whereas no significant alteration was detected on the *CuZnSOD* expression.

**RP139 Do land use patterns along Central Oregon rivers impact fish reproductive health and development?**

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Central Oregon is home to some of the fastest growing cities in the United States. Industry, agriculture, and tourism are rapidly increasing. The water quality and ecosystem health of the Deschutes River and its tributary, the Crooked River, are being impacted by increased anthropogenic contamination. *Gasterosterus aculeatus*, the Threespine Stickleback, is a widely used laboratory model for measuring the effects of water quality on vertebrate health. A naturally occurring stickleback population in Central Oregon rivers provides an exciting opportunity to harness the power of this lab model to test water quality issues in a natural setting. Do land uses associated with toxic and/or endocrine disrupting compounds- such as golf courses and urban storm water drainages – impact fish health in adjacent waterways? To address this question, we have measured concentration of the steroids 11-Ketotestosterone (11-KT) and 17β-Estradiol (E2), fish sex ratios, and gonad development in threespine stickleback from waterways adjacent to three main land uses: golf courses, urban centers, and protected watershed (control sites). Using standard pathohistology and endocrine ELISA methods, we have found that resident fish from waterways adjacent to golf courses have shown elevated levels of 11-KT. Interestingly, preliminary data show elevated vitellogenin production in males where 11-KT was also high, suggesting the presence of androgenic contaminant. We find increased spermatogenesis in male stickleback, and a decrease in oocyte production in females. Like humans, stickleback exhibit X Y sex determination systems. Preliminary sex genotype data have not identified any intersex individuals, but we are examining altered sex ratios in some populations from disturbed sites. Our initial findings suggest that patterns of disease and dysgenesis may emerge from our monitoring of sites along these rivers. These data provide preliminary support for using stickleback throughout Central Oregon as a “canary in the coal mine” for water quality. Next steps will include using yeast screens for estrogenicity and androgenicity on water collected from the Deschutes and Crooked River, and to examine the physiological and genetic mechanisms underlying the dysgenesis found in stickleback at some sites. We will discuss the broader implications of our findings in light of natural resource and urban growth management and mitigation of the effects of the rapid growth cities in Central Oregon.

**RP140 Does exposure to contaminants during early life stages impede recovery of the endangered copper redhorse?**

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The copper redhorse (*Moxostoma hubbsi*) is an endangered fish that exists only in the St. Lawrence River and its main tributaries in southern Quebec, Canada. According to recent estimates, copper redhorse are restricted to a single population consisting of at most 2,000 mature individuals. A myriad of anthropogenic factors may be leading to the poor natural recruitment of copper redhorse, including contamination of the only known spawning grounds and nursery habitats within the Richelieu River. Previous research on the effects of contaminants on copper redhorses has focused on adult fish and on legacy contaminants. Here, we focus our efforts on the early life stage and emerging contaminants. Water samples were collected from two known spawning areas, in December 2017 (n=2 per site). The purpose of this initial screen was to determine which contaminants might be present and of concern. A series of pharmaceuticals and personal care products (PPCPs), flame retardants, and pesticides were measured in the surface water samples. Preliminary results indicate that even in December when pesticides were not applied to surrounding croplands, some herbicides, such as atrazine (0.036 µg/L) and glyphosate (0.023 µg/L) were present in river water, although they did not exceed Quebec’s criterion for protection of aquatic life as they do in the spring. The glyphosate metabolite, aminomethylphosphonic acid, was also present (0.053 µg/L). The contaminant with the highest concentration detected was metformin (0.517 µg/L), a diabetes drug. At high concentration (40 µg/L), metformin has been linked to intersex and reduced fecundity in fish. In spring 2018, we deployed passive samplers at both sites (n=2 per site) to capture the temporal variation of pesticides during the spawning season, but also to determine their bioavailability. We also measured atrazine and glyphosate in river water collected from two tributaries and the Richelieu River spawning grounds. Building on previous results, we will rear copper redhorse eggs obtained from the provincial government artificial breeding program during the summer. Half the eggs will be constantly exposed to river water while the other half will be exposed to laboratory water. A suite of biomarkers will be measured to evaluate effects of exposure. This study will provide valuable data on the types of contaminants present in the Richelieu River and increase our knowledge on the potential effects they have on an endangered fish.

**RP141 Does growth efficiency influence mercury bioaccumulation in Largemouth Bass from Missouri reservoirs?**

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The Missouri Department of Health and Senior Services recommends that women of child-bearing age and children under 13 consume no more than one meal per month of large predatory fish due to elevated mercury (Hg). However, Hg concentrations in fish of the same size and species can differ depending on the reservoirs from which they were taken. We currently lack an understanding of the key factors contributing to variable Hg bioaccumulation. This has led to confusion regarding the safe consumption of fish. Previous monitoring efforts by the Missouri Department of Conservation reported that Hg concentrations in Largemouth Bass (*Micropterus salmoides;* LMB) were positively correlated with fish length and negatively correlated with reservoir surface area. Elevated Hg concentrations may also relate to body condition of fish and habitat quality, but these interactions have yet to be examined for LMB in Missouri. Muscle Hg concentrations of LMB (n=12/lake category) from 6 small (12 inches, PSD12 57-68) featured LMB that were in relatively good condition (Mean ± SD, 111 ± 40) and had the lowest concentrations of Hg (236 + 168 ppb; p< 0.001). Hg concentrations were highest (445 ± 247 ppb) and $W_r$ lowest (96 ± 34) for LMB in lakes that had a PSD12 < 40 indicating a stunted population. LMB from lakes with proportionately greater number of large fish (PSD12>70) had Hg concentrations and $W_r$ at intermediate levels (267 ± 150 and 97 ± 14, respectively). A more balanced population suggests that prey abundance and water quality conditions are favorable, and that fish may be limiting Hg bioaccumulation through growth dilution. Greater bioaccumulation of Hg may result from relatively slower growth and greater metabolic demands of LMB in stunted populations. These data will assist in decisions as to whether site-specific fish consumption advisories due to Hg are warranted, and whether fisheries management strategies could mitigate Hg bioavailability. This information will aid in the protection of fish consumers, and preserve the economic and recreational value of Missouri’s aquatic resources.

**RP142 Effect of heavy metals as important environmental pollutants**

*K. Adegbenro, University of Lagos, Nigeria*

The pursuit of industrailization results in the pollution of the environment. Analyzing the toxicological effect of pollutant in the environment, pollutant include ; persistent organic pollutant (POP), heavy metals and nutrient. i am concern about heavy metals because of thier common occurence and public health relevance. the key heavy metals are Mecury (Hg), Cadmium (Cd), Arsenic (As), Chromium (Cr), Lead (Pb), others include Zinc, Copper and Nickel. heavy metals are biacummulative and enter the environment through mining activities, industrial discharge and household electronic appliance and e-waste. They pose risk to human and animal even at low concentration. lead (Pb) causes damage to nervousse system, kidney and reproductive system, Cadmium (Cd) causes renal disfunction and bone degeneration, while Nickel which is used mainly in electroplating and causes harm to aquatic systems. Mecury (Hg) can cause deformity in new-born babies and acquatic bodies. Chronium (Vi) also causes liver and kidney damage and skin Ulceration. I therfore summit that toxic effect and poisoning by heavy metals world wide require strict regulations for discharge of solid waste and wastewater (affluents) into aquatic bodies.

**RP143 Effects of 17α-dihydrotestosterone injection on steroidogenic gene expression and plasma sex hormone levels in male mummichog (*Fundulus heteroclitus*)**

*R. Rutherford, A.L. Lister, D. MacLatchy, Wilfrid Laurier University / Biology*

The mechanisms of androgenic endocrine disrupting chemicals in fish are understudied compared to their estrogenic counterparts. Androgen exposure decreases plasma sex hormones, such as testosterone (T) and 11-ketotestosterone (11KT), in fish. Changes in gonadal steroidogenic gene expression may be linked to these depressions. However, in our previous in vitro bioassay of mummichog (*Fundulus heteroclitus*) testis tissue incubated in the presence of the model androgen 17α-dihydrotestosterone (DHT), there was an increase in steroidogenic gene transcript levels during 24 hours of exposure. This is counterintuitive to the decrease of plasma sex hormone levels we have observed in fish exposed to DHT. To further our understanding, an in vivo pilot study was undertaken, in which male mummichog were injected with 1 pg/g, 1 ng/g or 1 µg/g body weight DHT and sampled 6, 12, 18 or 24 hours post-injection to determine the time between injection and reduction of circulating sex steroids. Plasma T and 11KT were significantly depressed at 18 and 24 hours post-injection, in both the 1 ng/g and 1 µg/g treatments. No changes were observed at the 6 or 12 hour time points, or at any time in the 1 pg/g DHT treatment. Based on the pilot study, male mummichog were injected with 1 ng/g or 1 µg/g body weight DHT and sampled 15, 18, 24, 30 or 36 hours post-injection. Levels of steroidogenic gene expression were assessed at time points prior to and during plasma hormone depression. Genes included steroidogenic acute regulatory protein, 3β-hydroxysteroid dehydrogenase and cytochrome P450 17A1. By measuring gene expression levels at 3-6 hour intervals during the onset of plasma steroid depression, the time courses for plasma steroid depression and gene expression activity will be elucidated and indicate potential linkages.

**RP144 Effects of BPAP on thyroid hormone regulation and development in zebrafish embryos**

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Bisphenol A (BPA) analogues, such as BPAF, BPAP, BPF and BPS, have been widely used in various commercial products. Bisphenol AP (BPAP) has also been employed as an alternative developer in thermographic sensitive receipt paper. BPAP was detected in various environmental samples such as indoor dust, sediment and sludge and found in food and personal care products. Though endocrine disrupting potentials of BPA analogues (BPF and BPS etc.) were recently reported, the those of BPAP has not yet been well understood, especially on thyroid hormone. Since thyroid hormone is crucial for development and growth, this study was conducted to examine the effects of BPAP on thyroid hormone regulating system and development in zebrafish embryo/larvae model. To this end, zebrafish embryos (< 4 hr after fertilization) were exposed to BPAP (0, 0.04, 0.12, 0.37, 1.0 mg/L) until 144 hpf. Survival and hatching rate of zebrafish embryo was observed during the exposure. Then, 16 genes transcriptions related to thyroid hormone regulation (*crh*, *tshr*, *nkx2.1*, *hhex*, *tshβ*, *slc5a5*, *tg*, *pax8*, *tpo*, *trα*, *trβ*, *ttr*, *dio1*, *dio2*, *dio3* and *ugt1ab*) were analyzed. In addition, enriched gene ontology was identified based on RNA-sequencing. During the exposure, severe mortality was observed in the zebrafish embryos exposed to 1.0 mg/L BPAP. Hatchability and time to hatch was not affected up to non-lethal concentration (0.37 mg/L of BPAP). BPAP did not cause significant changes in gene transcriptions related to thyroid hormone regulation under non-lethal concentrations. Among the tested genes, only the transcription of *tg* gene showed significant increasing trend. Gene ontology enrichment related to thyroid hormone regulation was not found in zebrafish larvae after BPAP exposure. These results might indicate that BPAP was unlikely to affect thyroid hormone regulating genes and development under non-lethal concentrations. Thyroid hormone disrupting potency of BPAP might be relatively lower than that of other BPA analogues. This might be due to the structural differences (tri-ring sturucture) from other BPA analogues. A quantitative comparison among BPA analogues deserve further investigations.

**RP145 Effects of environmentally relevant concentrations of atrazine on early life stage in walleye (*Sander vitreus*)**

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The Richelieu River is an important breeding habitat for over half of the freshwater fish species found in Quebec, Canada, including walleye (*Sander vitreus*), pike (*Esox lucius*), and the endangered copper redhorse (*Moxostoma hubbsi*). Approximately 70% of the Richelieu's watershed is used for agriculture, and elevated concentrations of pesticides, including the herbicide atrazine, have been detected in surface water. Levels of atrazine in two of the main tributaries of the river are consistently within the range of concentrations shown to negatively impact fish health (>0.5 µg/L). In adult fish, atrazine exposure is known to negatively impact reproduction, but relatively few studies have focused on atrazine's impacts to early life stages of fish, particularly in ecologically relevant species. In the present study, walleye was chosen as a study species because of its presence in the Richelieu River, its distribution across northern United States and Canada, and the availability of eggs. Walleye eggs were obtained from a local hatchery and artificially reared in the laboratory matching environmental temperatures. Groups of 100 eggs were placed in plastic egg tumblers which use an air pump to keep the eggs agitated and aerated. Tumblers were placed individually in a glass aquaria containing environmentally relevant doses (0, 0.3, 3, 30µg/L) of atrazine dissolved in acetone (n=3 tumblers per dose for a total of 12 tumblers). The tumblers were effective in preventing the spread of fungus that had been problematic in previous experiments, however, hatching success was low with only 8% of embryos hatching; 84% of eyed eggs hatched suggesting that poor viability may have been related to unfertilized eggs. No significant difference in mortality was observed among treatments, however, atrazine-exposed groups hatched significantly earlier than controls. Twenty-four days post-fertilization, 39% of the high dose group (30µg/L), 13% of the medium dose group (3µg/L), and 3% of the low dose group (0.3 µg/L) had hatched. In comparison, none of the individuals in the control group had hatched. Future work will focus on deformities, DNA methylation (LUMA) and oxidative stress (TBARS) as endpoints. Preliminary results suggest that environmentally relevant levels of atrazine are non-lethal to larval fish, but can influence hatching time, which may negatively affect survival.

**RP146 Effects of hypoxia on the immune response of the eastern oyster (*Crassostrea virginica*)**

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Oysters are extremely important to marine ecosystems. From creating habitat for marine organisms to improving water quality through water filtration, oysters provide several vital ecosystem services. It is critical that efforts are made to maintain healthy oyster reefs to promote resilient marine ecosystems. In recent years however, a large reduction in oyster population has been observed. One study estimates that there has been an approximately 85% reduction in oyster reefs worldwide. Several factors including overharvesting and poor water quality conditions are thought to contribute to this reduction. Hypoxia (dissolved O2 < 2 mg/L) is a another contributing factor adversely impacting oyster reefs. Hypoxic events caused indirectly by algal blooms create a low oxygen environment that can be extremely harmful to marine organisms. In this experiment, subsets of oysters are exposed to differing lengths of hypoxic conditions (3 ,6 , and 9 days) followed by a 5 day recovery period in normoxic conditions (dissolved O2 > 8 mg/L). Oysters were sampled every other day and gill tissue was extracted to observe differential gene expression of immune related genes, thymosin-beta 4 (Tβ-4) and heat shock protein 70 (HSP-70). Additionally, expression of calmodulin and hypoxia inducible factor 1-alpha (HIF1-α) were analyzed by qPCR. Total hemocyte counts were measured to further assess the effects of hypoxia on oyster immune function. The results from the this laboratory study will be compared to those from field deployed oysters in the Mississippi Sound. This work will help determine effects of hypoxia on oyster's immune function and will contribute to the selection of optimal sites for oyster reef restoration efforts. This project is funded by MBRACE (Mississippi Based Restore Act Center of Excellence)

**RP147 Effects of Metformin on Growth and Appetite in Juvenile Fathead Minnows (*Pimephales promelas*)**

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Pharmaceuticals and personal care products (PPCPs) characterize a large class of chemicals that leach into the environment through human use and excretion. Recent quantifications of PPCPs in wastewater treatment effluent samples indicate that conventional treatment methods are ineffective at removing many of these chemicals, thus making PPCPs popular subjects of toxicological studies in the past few decades. Recent studies have shown that some pharmaceuticals can impact behaviors such as reproduction, predator avoidance, and food acquisition in some aquatic species at concentrations found in effluent wastewater. This study focuses on the effects of metformin on the growth and appetite of juvenile fathead minnows (*Pimephales promelas*). Metformin is a commonly prescribed medication used to treat type 2 diabetes, metabolic syndrome, infertility, and more. In this study, juvenile fathead minnows were exposed for seven days to three concentrations of metformin: a negative control, an environmentally relevant concentration (1.6 µg/L), and a positive control based on recently published studies (40 µg/L). We observed a significant change in appetite when the minnows were dosed with 40 µg/L of metformin (p=0.00295). However, we did not observe any significant changes in weight gained in any dose at the end of the experiment. It is possible that there may be changes in weight if the exposure period was longer than 7-days. These results suggest that further research is warranted to investigate the connection between metformin exposure, appetite, and growth in juvenile fathead minnows.

**RP148 Enantioselective toxic effects of 2, 2', 3, 5', 6 polychlorinated biphenyls (PCB-95) on anxiety-related behaviors in larval zebrafish**

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2, 2', 3, 5', 6 Polychlorinated biphenyl (PCB-95) is a non-dioxin-like, chiral PCB congener that has been identified as a potent neurotoxicant. Our previous study with racemic (rac-) PCB-95 demonstrated altered behaviors on swimming speed, thigmotaxis and avoidance in *Danio rerio* (zebrafish) at environmentally relevant concentrations. But the differential actions of individual atropisomers of PCB-95 on biological targets have not been studied. In the present study, PCB-95 enantiomers were separated using high performance liquid chromatography (HPLC) and assigned as (+) aR- and (-) aS-PCB 95 to the first and second eluent, respectively. Embryos at the two-cell stage with intact chorions were exposed to different concentrations of racemic and individual isomers of PCB-95 (0.25,0.5 0.75, 1ppm) with two controls (E2 solution and the DMSO). Exposure time was 3 days with 4 days incubation period. At day 7, anxiety-related behaviors were measured with a behavioral assay. Influences on mortality rates, hatching rates and malformations were also observed. Studies on mortality, hatching and malformation indicated that (+) aR-PCB 95 was more potent than the rac-PCB 95 and the (-) aS-PCB 95. The results showed a dose dependent increase in mortality and malformation rates but a decrease in the hatching rates in the (+) aR-PCB 95 and rac- PCB 95 treated groups. These results were further confirmed with the behavioral assay data. Significantly lower swim speed, enhanced thigmotaxis and lower avoidance rates were observed for the (+) aR-PCB 95 compared to the others. In conclusion, enantioselective toxicity of PCB-95 towards the behavior of an organism that links to the developing neuronal networks were investigated and may clarify health risks associated with enantioisomeric enrichment of PCBs in the environment.

**RP149 Evaluation of Nickel-Induced Genotoxicity and Histopathological Alterations in the Liver of *Clarias gariepinus* Burchell 1822 Post-juveniles**

*O. Adebolu, O.T. Aladesanmi, Obafemi Awolowo University / Institute of Ecology and Environmental Studies; M.F. Awodiran, Obafemi Awolowo University, Ile-Ife / Department of Zoology*

Behavioural changes, possible histopathological alterations and effects of nickel on the liver DNA of the exposed fish was evaluated in this study. A range finding test was carried out prior to the definitive test to determine the 96 hour median lethal concentration ($LC_{50}$) of Nickel Chloride. The experiment consisted of two control tanks (each for the male and female set-up) and four (10, 20, 30 and 40 mg/L) acute concentrations of nickel in duplicate with each treatment containing five fishes per tank. Behavioural changes were observed 12 hourly, for 4 days. At the end of the 4-day exposure, two fishes per concentration were randomly selected and their livers excised for histopathological analysis. Genetic analysis was performed, using the Random Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR) while heavy metal analysis was carried out on these liver samples, using the Atomic Absorption Spectrophotometry. All the treated fish exhibited mild to severe behavioural abnormalities such as unusual and erratic swimming, irregular opercula movements, gulping of air, restlessness, bending and loss of equilibrium. The $LC_{50}$ value for the acute toxicity was 21.45 mg/L. The histopathological examination of the liver samples revealed alterations such as dialated sinusoids that are not radially apparent, dialated central veins, numerous intracytoplasmic vacuolations, distorted histoarchitecture as well as lyphmphocytic infiltrations. The RAPD analysis showed DNA damage in the liver of treated fish samples compared to the control. Electrophoretic images revealed bands mostly from 200 to 800 bp and more bands were revealed in female fish samples as compared to the male fish samples. The study concluded that Nickel Chloride altered normal behavioural patterns, caused several histopathological alterations and DNA damage especially in female *C. gariepinus.*

**RP150 Histological and behavioral evaluation of the livebearer *Jenynsia multidentata* exposed to glyphosate: A comparative analysis of Roundup® formulations**

*C.D. Martins, J.A. Albañil Sánchez, Federal University of Rio Grande / Biological Sciences*

Roundup formulations are herbicides whose active principle is glyphosate. They are also composed of other ingredients, such as surfactants that are more toxic than pure glyphosate to fish. However, there are few studies comparing the effects of different formulations commercialized worldwide. Therefore, this study aimed to evaluate and compare the toxic potential of the Roundup formulations through histological and behavioral alterations in fish. For that, males and females of the neotropical fish *Jenynsia multidentata* were acutely exposed to Roundup Original® (RO), Roundup Transorb® (RT) or Roundup WG® (RWG), at a fixed concentration of 0.5 mg/L of glyphosate. This concentration is close to the maximum glyphosate limits found in the environment and is non-lethal to *J. multidentata*. The three formulations caused histological damage to the liver, gills and brain of *J. multidentata*, which increased over the exposure time (24 to 96 h). Females were more tolerant to RO, RT and thereafter to RWG, respectively. Males did not exhibit these differences in sensitiveness with formulations. The RWG caused more damage in liver and gills and RT in the brain. Behavior tests performed after 96 h of exposure were: open-field test, social interaction, aggressiveness, and long-term memory (LTM) tested in an inhibitory avoidance apparatus. Results showed that the RO, RT and RWG formulations caused neurotoxicity, reaching behavioral patterns. It was observed that *J. multidentata* present a natural anxious conduct that significantly increases under RWG exposure. This formulation also caused a decrease in social interaction of livebearer. On the other hand, RO caused depression in the animals more than other formulations. The LTM was negatively affected by the presence of RT and RWG. Overall, RWG formulation was the most aggressive for *J. multidentata* exhibiting a more diffuse action, whilst the RT seems to have the central nervous system as a target-organ. The results reinforce the potential use of the *J. multidentata* as an indicator of the presence of agrochemicals. Differences between formulations toxicity and their effects are directly related to the inclusion of other components besides glyphosate, which are described in their respective labels as "inert" compounds. The comparison of the toxic potential of glyphosate-based herbicides is important to give support to the governmental organizations to set protective rules for the ecosystems and for human health.

**RP151 In vitro cytotoxicity of B[a]P, PCB 77, PCB 126, and PFOA in gray whale (*Eschrichtius robustus*) skin fibroblasts**

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The gray whale (*Eschrichtius robustus*) is long-lived and migratory, often traveling and feeding close to dense coastal human populations. Under the MMPA, separate eastern and western North Pacific stocks are recognized, and the small WNP stock is listed as Endangered under the ESA. Persistent marine contaminants such as polychlorinated biphenyls (PCBs) (historically produced for industrial purposes), polycyclic aromatic hydrocarbons (PAHs) (produced from the burning of fossil fuels and wood), and per/poly-fluorinated compounds (PFCs) (found in stain repellents and anti-stick cookware) have been detected in cetacean blubber. However, little is known about the effects of these contaminants in gray whales. We aim to understand whether organic pollutants may be cytotoxic in gray whale tissue and whether these toxicants are of concern for the species. Due to their protected status, cell culture is invaluable as it is minimally invasive and allows for multiple investigations using a single biopsy. We cultured primary fibroblasts from skin biopsies obtained from three free-swimming gray whales off the coast of California. Each biopsy was minced and placed under a coverslip in a gelatin coated cell culture dish. Cells were cultured in DMEM/F12 media supplemented with 15% cosmic calf serum, 1% penicillin/streptomycin, 1% glutamax, and 0.1% sodium pyruvate. Cells were incubated at 37°C in a humidified atmosphere of 5% $CO_2$. We analyzed cytotoxicity via MTT (methylthiazolyldiphenyl-tetrazolium bromide) and LDH (lactate dehydrogenase) assays. Toxicants selected for our study were benzo[a]pyrene (B[a]P) or PCB 126 or PCB 77 in concentrations of 10µM, 1µM, 0.1µM, or 0.01µM or lastly perfluorooctanoic acid (PFOA) in concentrations of 500µM, 50µM, 5µM, 0.5 µM, or 0.05µM . Exposure time points were 24h, 48h, 72h, and 96h for each toxicant. Cytotoxicity was determined colorimetrically via spectrophotometer for both MTT and LDH assays. Preliminary results indicate that cellular viability was significantly reduced after exposure to B[a]P, PCB 126, PCB 77, and PFOA at various concentrations and time points. Additional data analyses are underway.

**RP152 In vivo quantification of xenobiotic metabolism in freshwater snails**

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Mixed function oxygenases (MFOs) and esterase enzymes comprise an organism's first line of defense against xenobiotics. MFOs exhibit broad substrate specificities, which allow them to alter/detoxify a wide variety of endogenous and exogenous compounds. Consequently, MFO and esterase activity plays an important role in dictating sensitivity to xenobiotics. The current study aims to validate an in vivo assay that quantifies metabolic activity in response to multiple MFO and esterase substrates simultaneously in freshwater snails (*Helisoma* and *Physa* spp.). Organisms were exposed to 6 µM concentrations of four substrates—4-nitroanisole (4-NA), 7-ethoxycoumarin (7-EC), 7-ethoxyresorufin (7-ER), and 1-naphthyl acetate (1-NpA)—for six hours. Subsequently, the metabolites of these substrates were extracted from the experimental matrix via supportive liquid extraction. The analytes within each sample were separated and quantified using an LC-MS, after which a "metabolic profile" was constructed. This non-destructive assay permits a comprehensive evaluation of xenobiotic metabolism that could facilitate population-level assessments of contaminant sensitivity.

**RP153 Inter-generational impacts of crude oil exposure on genome-wide expression in developing Gulf Killifish embryos**

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A growing body of research demonstrates that biological impacts of environmental toxicants can persist across generations. Oil spills are of ecotoxicological concern, because they occur frequently, impact large geographic areas, affect diverse species, and can occur in sensitive habitats. Much research has sought to reveal impacts of oil exposure on development and physiology, including its underlying mechanisms. In contrast, relatively little is known of how toxicity could persist across one or more generations. We characterized the developmental and inter-generational impacts of laboratory crude oil exposure in Gulf killifish using transcriptomic and physiological endpoints. After exposing adult killifish to weathered crude oil and control conditions, we tested whether offspring from exposed parents had altered gene expression and sensitivity to oil toxicity during embryogenesis. We found that parental oil exposure disrupted developmental gene expression patterns in their offspring, and identified molecular responses that are potentially involved in mechanisms of intergenerational crude oil toxicity.

**RP154 Investigation on oxidative and genotoxic stress effect of heavy metals and pesticides exposure on tardigrade and the antioxidative effect of vitamins**

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The exhaustive use of chemicals, like pesticides, heavy metals and some drugs leads to chronic exposure to lower doses of these toxicants. Cadmium and copper are highly toxic environmental contaminants, though cadmium is more toxic. According to literature, these metals form a serious hazard to the public health and a threat to most life forms. Cadmium and copper are potent toxicants that are commonly found in aquatic environment as a result of anthropogenic activities (Hygum et al., 2017). Studies have shown Copper to inhibit osmoregulatory enzymes and induce oxidative stress, while Cadmium inhibit cell proliferation due to its carcinogenicity. Human genotoxicity and carcinogenicity due to exposure to insecticide represent a prominent public health hazard. Mammals utilize specific pathways like apoptosis to ameliorate or reduce the resulting hazard from DNA damage. (Bock et al., 2015). Lufenuron and Chlorfluazuron are both pesticides. Exposure to these pesticides have been studied to have adverse effects like, genotoxic stress and reproductive inhibitors, respectively. Tardigrades are microscopic aquatic animals renowned for their tolerance towards extreme environmental conditions, this study will investigate their tolerance towards heavy metals and pesticides and how to ameliorate these effects with antioxidants (vitamins). Antioxidants are substances that may protect the cells against the effects of free radicals, they are intimately involved in the prevention of cellular damage which is the common pathway for cancer, aging, and a variety of diseases. In the laboratory, tardigrades will be exposed to varying concentrations of heavy metals (cadmium and copper) and pesticides (Lufenuron and Chlorfluazuron) by simulating the environmental route of exposure. Genomics combined with culturing may identify tardigrades that may be tolerant to these extreme conditions. After discovering these effects on the tardigrade cells, the tardigrades will be treated with antioxidant (vitamins) which has the ability to sequester the heavy metals and prevent free radical formation by the pesticides. I expect to gain a better understanding of the mechanism of action of heavy metals and pesticides in the formation of free radicals, genotoxic stress, inhibiting reproduction and inducing carcinogenic response. Finally, I hope to develop an understanding of how vitamins can be used to ameliorate these adverse toxic insults induced by pesticides and heavy metal exposure.

**RP155 Modeling the effect of branching on the aquatic ecotoxicity of surfactants and its application for effect assessment and testing decisions**

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Surfactants play important roles in consumer product formulations including laundry and dish detergents, shampoos, and conditioners. Environmental fate and effects of those surfactants have been studied extensively providing a long history of safe use. Linear and essentially linear surfactants have been worked most intensively with ecotoxicological tests and mesocosms leading to well established aquatic predicted no-effect concentrations (PNEC). For example, the anionic surfactant category of alcohol ethoxy sulfates (AES), PNECs are based on species sensitivity distributions using chronic effect and mesocosm data normalized to an environmentally relevant structure. However, for branched surfactants which can meet biodegradability requirements, assessment and predictive tools are greatly needed as much less testing data is available. Conventional wisdom suggests branched surfactants should be less toxic to aquatic species than essentially linear ones, as branching increases the hydrophilic nature of surfactant. This work intends to quantify ecotoxicity of branched surfactants by quantitative structure activity relationship (QSAR) modeling and validate the approach using new ecotoxicity data. The modeling methodology started with calculating log P for individual components of surfactant mixtures employing a position-dependent branch factor, then determining acute and chronic toxicity based on QSAR for each component, lastly deriving entire mixtures' toxicity by a toxic unit approach. Examples of aliphatic branched AESs were provided with model prediction and recent experimental results on the invertebrates *Daphnia magna* and *Ceriodaphnia dubia*. The majority of the ratios of model and measurement of effective concentrations (EC50) fell within a factor of three with less variability on more toxic compounds. By integrating literature data, 60% of model predictions were within two-fold of measured values and 80% within a factor of three. One of the applications for this modeling work was to quantify mixture toxicity differences of branched versus linear surfactants. The case study suggested that for lesser branched AESs, ecotoxicity difference is not significant, however, for highly branched, noteworthy reduction for ecotoxicity could be expected (e.g., acute EC50 increase of 60-80%). The findings are currently being applied for effect assessment, aiming prioritization and reduction of ecotoxicity testing.

**RP156 Molecular biological analysis of biodegradation behavior in the ready biodegradability test**

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Ready biodegradation test OECD TG 301 has been required under the Japanese Chemical Substances Control Low (JCSCL) for chemical safety management. The test shall be conducted at a quite high concentration and hence, bioavailability to microorganisms is too low to reflect a biodegradation profile in an actual environment especially for poorly water soluble chemicals. In addition, the test shall be done with the standard microbial inoculum cultivated in a synthetic sewage containing mainly glucose and peptone for at least one month and consequently, the inoculum has a lower degradation activity than that in an actual environment, leading to false negative outcomes especially among ready biodegradation tests. Therefore, ready biodegradation test OECD TG 301F has been introduced under JCSCL since April 2018, in which an inert carrier or an emulsifying agent can be used to improve bioavailability to microorganisms and a microbial inoculum taken from a treatment plant receiving predominantly domestic sewage can be used to reflect degradation activity under an actual environment. Nonetheless, there are few studies to clarify the influence of an inert carrier or an emulsifying agent on flora of the degrading microorganisms or to investigate differences in microflora between the standard microbial inoculum and the inoculum taken from a sewage treatment plant. In this study, a silica gel and Tween 80 were used as an inert carrier or an emulsifying agent, respectively, to improve chemical dispersity. Dibenzofuran, anthraquinone, 2-ethylanthraquinone and tris(2-ethylhexyl)trimellitate were used as poorly water soluble chemicals classified as not readily biodegradable under JCSCL. One hundred mg/L of each chemical was exposed to 30 mg/L of the standard microbial inoculum or the inoculum taken from a municipal sewage treatment plant, and microbial DNA was extracted from the degrading microorganisms when remarkable biodegradation was observed in monitoring the biochemical oxygen demand curve or HPLC analysis, and then the microflora was investigated by a metagenomic analysis of bacterial 16S rRNA (V3-V4 region) using next-generation sequencer. Several cases of variations in microbial community structure occurred during the biodegradation, and further analyses were conducted to determine dominantly increasing microorganisms. These results are reported in this presentation.

**RP157 Molecular, Physiological and Behavioral Responses of Embryo-Larval Zebrafish Exposed to Types I and II Pyrethroids**

*O.M. Awoyemi, J. Crago, Texas Tech University / Environmental Toxicology / The Institute of Environmental and Human Health (TIEHH)*

Pyrethroids are highly toxic to aquatic species. Most toxicity studies assess only single chemicals, which makes it difficult to assess relative toxicity across classes of pyrethroids. There are limited studies comparing the developmental toxicity of type I and II pyrethroids in aquatic systems at environmentally relevant concentrations. The goal of this study was to compare the molecular, physiological and behavioral effects of pyrethroid exposures in larval zebrafish (Danio rerio). Zebrafish embryos were exposed to type I (bifenthrin, permethrin) and type II (deltamethrin, $\lambda$-cyhalothrin, fenvalerate, esfenvalerate) pyrethroids at 1000, 10, 0.1, 0.01 µg/L starting at 5 h post-fertilization (hpf) through 5 d post-fertilization (dpf). Behavior assays assessing swimming behavior (distance traveled and velocity) were conducted at 5 dpf. The stability of pyrethroids across the 5 days was analyzed using LC-MS/MS. Gene expression using RT-qPCR, total reactive oxygen species (ROS), and enzymatic activities were determined to assess relative toxicity amongst the pyrethroids. The initial concentrations of all six pyrethroids were significantly ($p< 0.05$) reduced at days 3 and 5 of the exposure timeline. However, both the type I and II pyrethroids had significant ($p< 0.05$) effects on the behavior of the zebrafish larvae, as compared to control. Type I pyrethroids demonstrated a U-shaped dose response with behavioral endpoints at 5 dpf, whereas type II pyrethroids showed no specific pattern in dose response with behavioral endpoints as compared to the control. The type I and II pyrethroids had significant ($p< 0.05$) effects on the total ROS production, as well as the expressions of certain genes including Nrf2a, Cas9 and p53, which were indicative of oxidative stress in the zebrafish embryo-larval development. Further studies are being carried out to assess the enzymatic activities in zebrafish exposed to these pyrethroids.

**RP158 Morphometric Effects of Various Microplastics on Sac Fry Zebrafish**

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Microplastics (1nm to 5mm) and plasticizers are ubiquitous in waterways, beaches, sediments and biota across the world. It is estimated that since the emergence of plastic in the 1950s, ~18 trillion pounds of plastic have been produced. Ingestion of microplastics by various marine species and accumulation of plasticizers has been documented and is of growing concern. Additionally, microplastics act as a carrier for the transport of persistent organic pollutants, increasing the hazard to aquatic species. Microplastics vary in composition based on its monomeric component and the specific plasticizer, i.e. chemical additives that increase the plasticity or viscosity of plastics. The toxic effects associated with the components that comprise the polymerized plastic are not well characterized. The results presented here examine gross morphological variations in sac fry zebrafish as a result of exposure to weathered microplastics and pure plastic polymers. Embryos were exposed from 3 hours post fertilization (hpf) to 96 hpf with samples of weathered microplastics from Newark Bay, NJ, as well as commercially available pure plastics at concentrations of 1 µg/mL and 10 µg/mL. The Newark Bay microplastics were analyzed via pyrolysis GC-MS to determine composition. Pyrolysis GC-MS determined that the three samples were composed primarily of polyethylene (PE), polypropylene (PP) and polyvinyl chloride (PVC). Morphometric data were statistically analyzed, and no significant change was noted between the controls and the treated groups in the embryonic Zebrafish samples for the Newark Bay, Fenton treated, weathered samples. The commercial microplastics tested included: PE (low, medium and high density), polystyrene (PS), PVC, sodium polyacrylate (SPA), polyethylene terephthalate (PET), polyurethane (PU), and polymethyl methacrylate (PMMA). Significant changes were seen in total body length in the polyethylene 10 µg/mL exposure group. Significant changes were seen in pericardial sack size in polyethylene low density (LD) 10 µg/mL, polyethlene high density (HD) 1 µg/mL and 10 µg/mL, and polystyrene 1 µg/mL and 10 µg/mL exposures. Preliminary gross observations show pericardial sac edema as a common endpoint. (NJAES #01201)

**RP159 Nephrotoxic effects of glyphosate and Rodeo ® through water exposure on largemouth bass**

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Glyphosate is the most widely used non-selective broadleaf herbicide in the world including Florida. It has been used extensively for weed control on non-croplands, with glyphosate tolerant crops and a ripener for sugarcane. Farmers chronically exposed to glyphosate have shown unusual kidney disease. Glyphosate can be found in waterways as run off from crops or be directly sprayed into water bodies to control overgrowth of aquatic vegetation (Rodeo® formulation). Glyphosate can persist for 60-70 days in water and it has also been detected in the ocean. With its extensive use and slower than expected degradation, aquatic animals can be chronically exposed to glyphosate. Long-term exposure experiments with animal models, like fish, have shown reproductive impairments and kidney disease. The toxicity mechanism is through the formation of reactive oxygen species. Our objective is to assess kidney damage in largemouth bass after chronic exposure to glyphosate and Rodeo®. We exposed male individuals (n=4/tank, 4 replicates/treatment) to 0.5 and 10mg/L concentrations of glyphosate and Rodeo® (EPA Reg. No. 6219-324, chemically equivalent concentration) in water for 28 days. Our lowest concentration was similar to the EPA maximum concentration allowed for drinking water. Fresh exposure solution was made every 5 days, and 70% of the tank water was changed daily. Actual concentrations of glyphosate in exposure solutions were verified by LC-MS/MS. Our preliminary histopathology results, show apoptosis and necrotic tissue in the high-dose treatment and not in the control. We will analyze changes in gene expression through RNA sequencing of the trunk kidney. We expect to detect upregulation of genes related to kidney damage such as kidney injury molecule (KIM-1), beta-2-microglobulin and neutrophil gelatinase-associated lipocalin (NGAL) in the higher-doses in comparison to control. We expect to detect enriched gene pathways associated with apoptosis and oxidative damage. Our research will identify possible molecular markers for kidney damage as a consequence of exposure to glyphosate or Rodeo® in fish that can be extended to other species exposed through their environment.

**RP160 Neurobehavioral effects of organophosphates on embryo-larval zebrafish at environmentally relevant concentrations**

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Organophosphates are common surface water contaminants in both urban and agricultural landscapes. Neurobehavioral effects on larval fish are known to occur at concentrations higher than those reported; however, behavioral effects have yet to be investigated at environmentally relevant concentrations. For this ongoing study, the developmental neurobehavioral effects of organophosphate exposure on embryo-larval zebrafish (*Danio rerio*) were assessed using a larval zebrafish behavior assay. Five common organophosphates (chlorpyrifos-oxon, methyl-parathion, malathion, dichlorvos, and diazinon) were assessed individually at environmentally relevant concentrations. Embryos were exposed to chlorpyrifos-oxon at 1mg/L, which resulted in fatality of all embryos. Subsequent embryos were exposed to organophosphates at 100 µg/, 10 µg/L, 100 ng/L, and 10 ng/L starting at 4 h post-fertilization (hpf) to 5 d post-fertilization (dfp). Behavior assays assessing swimming behavior (distance traveled and velocity) were conducted at 5 dpf. Data was analyzed using one-way ANOVA with Tukey HSD post hoc test. Zebrafish larvae were tested individually at 100 µg/L chlorpyrifos-oxon ($p < 0.05$) and 100 µg/L diazinon ($p < 0.05$) and data revealed that zebrafish larvae traveled a significantly lesser distance than the controls. All chlorpyrifos-oxon treatment groups travelled lesser distance than the control group. Velocity of larva exposed to 100 µg/L chlorpyrifos-oxon and 100 µg/L diazinon decreased significantly compared to their control groups. Preliminary chlorpyrifos-oxon and diazinon data indicates behavioral changes at 100 µg/L in larva. All organophosphates displayed a decreasing trend in distance traveled and velocity relative to their controls. Relative expression of AChE mRNA indicated a downward trend in fold change for all organophosphates, and acetylcholinesterase (AChE) activity assessments indicated a downward trend for chlorpyrifos-oxon and malathion. Ongoing carboxylesterase (CES) assessments suggest an upward trend in activity for chlorpyrifos-oxon, methyl-parathion, and malathion. Investigation into the neurobehavioral effects of organophosphates' continues.

**RP161 NPDES Whole Effluent Toxicity (WET) Toxicity Identification Evaluation/Toxicity Reduction Evaluation (TIE/TRE) Poster Abstract**

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Under the National Pollutant Discharge Elimination System (NPDES) permits program, the use of aquatic toxicity testing such as whole effluent toxicity (WET), along with Toxicity Identification Evaluations and Toxicity Reduction Evaluations (TIEs/TREs) where necessary, have been instrumental in addressing identified toxic impacts to aquatic life. The focus of this presentation is to provide case examples of TIEs/TREs from approximately the last five years, conducted using the recommended approaches in Environmental Protection Agency's (EPA) TIE/TRE guidance documents. The examples are derived from actual NPDES TIE/TRE cases which were successful in reducing or eliminating sources of toxicity. A brief list of key recommendations from EPA's TIE/TRE procedures will be provided and further illustrated by the case examples, which include descriptions of some of the diagnostic approaches used to further assess ambient or effluent toxicity data that indicated excursions of state aquatic life water quality standards. In this presentation, we will provide an overview of some of the important scientific principles, key technical considerations and EPA recommendations that should be considered when conducting TIEs/TREs. The presentation will cover the primary advantages of a well-planned and executed TIE or TRE, especially for resolving possible impacts to aquatic organisms and for making decisions about water quality protection of aquatic life. We will discuss how TIEs/TREs can provide valuable insights into the causes and sources of toxicity as well as some recommended approaches for resolving ecological impacts to aquatic communities due to exposure to pollutants. *The views expressed in this presentation are those of the author(s) and do not necessarily represent the views or policies of the Agency.*

**RP162 Partitioning of azithromycin into Chinook salmon eggs from American River water**

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Azithromycin (AZ), a potent antibiotic and USEPA Emerging Contaminant of Concern, has been found in aquatic systems all over the world. After release into the environment through sewage effluent, AZ can be found in both soil and water where organisms can be exposed, however very little is known about AZ in aquatic systems. California river systems are integral to the economy as well as vital for endangered species like salmonids. Water and conditions from the American River, a spawning ground for salmonids such as the Chinook salmon (Oncorhynchus tshawytscha), were used in this study to represent California river systems. Winter run Chinook salmon eggs (6.01 ± 0.02 g) were shaken in microcosms consisting of 200 mL of filtered American River water at 10°C ± 1 for 24 h. After equilibration, microcosm water was dosed with 5.32 x 10?? µM ¹?C radiolabeled AZ plus 5.34 x 10?¹ µM unlabeled AZ. Microcosms were sacrificed at 0, 4, 8, 16, 25, and 49 h, at which times eggs, water, and glass sorption were measured. Samples were analyzed via Liquid Scintilation Counter. The concentration of AZ sorbed to the eggs reached equilibrium after 25 h. After 49 h, less than 1% of the original dose of AZ had partitioned into the eggs from the surrounding water. Ratio of egg mass to water volume was explored using varying egg masses (3.02 ± 0.01, 12.00 ± 0.02, and 16.01 ± 0.01 g). The eggs were exposed as above and measurements taken at 48 h. A linear trend was observed between egg mass and the total AZ sorption into the eggs ($R^2$ = 0.95). Based on this study, AZ does not readily sorb to salmon eggs, despite having a high potential to concentrate in tissue.

**RP163 Reconstructing trace element exposure histories of fish using otolith microchemistry in watersheds that contain energy-extraction industry activity**

*N. Bailes, The Ohio State University / Environmental Science Graduate Program; R.P. Lanno, Ohio State University / Department of Evolution, Ecology, and Organismal Biology*

The proliferation of unconventional oil and gas wells that utilize high volume horizontal hydraulic fracturing (HVHHF) technology has yielded enormous quantities of highly saline produced waste waters. Despite the implementation of various safety protocols, releases of these waste waters into surface waters has occurred. In the absence of continuous monitoring of baseline water chemistry, it is difficult to quantitatively assess the exposure of aquatic organisms to contaminants originating from HVHHF processes. Analysis of fish otoliths is one potential method for quantitatively assessing contamination exposure in the absence of continuous monitoring. We collected sunfish (*Lepomis* sp.) from various watersheds throughout eastern Ohio. Each site was selected based on its proximity to energy-related resource extraction activities. Fish were sampled from watersheds that encompass surface coal mining operations, sites of acid mine drainage, HVHFF activities, as well as a reference watershed where no ongoing energy-related resource extraction activities had taken place. Through laser-ablation inductively-coupled plasma mass spectrometry (LS-ICP-MS), we characterized otolith trace metal content ($Li^{7}$, $Na^{23}$, $Mg^{24}$, $Mg^{25}$, $As^{75}$, $Sr^{88}$, $Cd^{111}$, $Ba^{137}$, $Ba^{138}$, $Hg^{200}$, $Pb^{208}$, $S^{32}$, $Cr^{52}$, $Fe^{56}$, $Ni^{60}$, $Zn^{64}$) for fish from each of these sites in order to explore the potential of these data to discriminate between the trace element signatures associated with various of energy-related resource extraction processes. We will also discuss the relationships between accumulation of the different metals over the lifespan of the sunfish and over the time of development of new extraction industries in the watershed.

**RP164 Responses to Hydroxychlorothalonil and Dicloran Exposure by *Menidia beryllina* in the Presence of Varying Salinities and Sunlight**

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Hydroxychlorothalonil is the primary soil degradation product of the fungicide, chlorothalonil, which is applied to crops, turf, and ornamentals; unlike chlorothalonil, hydroxychlorothalonil is readily re-suspended into the water column and can undergo photodegradation in the presence of sunlight. Dicloran is a systemic fungicide applied to a variety of crops throughout the western and southern United States including celery, lettuce, and sweet potatoes; it also can undergo photolysis degrading through a quinone-hydroquinone pathway. Both fungicides have similar reported photodegradation pathways, intermediate degradation products, and differences in distribution between seawater and freshwater. Inland silversides (*Menidia beryllina*) can be found throughout North America and have a wide tolerance of salinities. Using varying salinities, the differences in the response to potential toxicity and phototoxicity of dicloran and hydroxychlorothalonil can be observed in inland silversides. At concentrations as low as 0.10-mg/L dicloran, sub-lethal impacts such as an upregulation in the gene expression of silversides can be observed at salinities of 25 ppt; at concentrations as high as 0.50-mg/L, total mortality can be observed in the presence of sunlight. At 12 ppt salinity, total mortality can be observed at concentrations as low as 0.10-mg/L dicloran in the presence of sunlight, while 0.17-mg/L dicloran showed roughly 80% mortality at salinities of 5.5 ppt.

**RP165 RNA-sequencing helps understanding the effects of benzotriazole UV-stabilizers in dietary exposed juvenile rainbow trout (*Oncorhynchus mykiss*)**

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Phenolic benzotriazole UV-stabilizers (BZT-UVs) are widely used as additives to protect from light-induced yellowing and degradation in a variety of products (e.g., paints, coatings). Despite their widespread presence in aquatic ecosystems and persistence in the environment, information on the effects, toxicity or modes of action of these compounds remain largely unknown. The objectives of the present study were to evaluate the chronic effects of two BZT-UVs, 2-(2H-benzotriazol-2-yl)-4,6-bis(1-methyl-1-phenylethyl)phenol (UV-234) and 2-(2H-benzotriazol-2-yl)-4,6-di-tert-pentylphenol (UV-328), in juvenile rainbow trout chronically exposed (28-d) through diet. Chemical analyses of livers from exposed trout showed a higher accumulation of UV-328. Additive and synergistic effects of the UV-328 and UV-234 mixture were also observed on the accumulation of each compound in the liver (i.e., 6 and 2 times the nominal concentrations in the amended feed, respectively). A 120-h depuration period at the end of the 28-d exposure showed similar and limited depuration rate of 25.9 and 28.3% per day for UV-234 and UV-328, respectively. RNA-sequencing analyses of exposed trout liver were used to identify the transcriptional effects of BZT-UVs individually or in a mixture. Results revealed specific effects of each compound on gene transcription profiles; UV-234 affected mainly genes involved in cellular metabolism (cholesterol, glucose, energy), while UV-328 induced the transcription of many ribosomal proteins and down-regulated the transcription of genes involved in immune responses. Additive effects of the mixture of both BZT-UVs were observed on the regulation of two immune-related genes. Quantitative real-time PCR measurements confirmed the effects of UV-234 and UV-328 on the transcriptional regulation of a suite of candidate genes. Further analyses of related protein activity will help identify potential biomarkers of exposure and characterize the modes of action of BZT-UVs in fish.

**RP166 Suitability of effect-based bioassays for mixture effect elucidation**

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In the environment, chemicals occur in complex mixtures rather than individual entities. Hence, environmental quality monitoring faces the challenge to comprehensively assess a multitude of contaminants and potential adverse effects. To meet this challenge, effect-based methods have been suggested as complements to analytical characterization of complex pollution patterns. Here, we present an interlaboratory study which investigates the suitability of effect-based bioassays for mixture effect elucidation by conducting the toxicological potential of individual, high priority pollutants and their mixtures with a panel of effect-based bioassays. The tested compounds were of diverse chemical structure and unique known mode of action (MoA). The bioassay panel consisted of whole organism assays measuring apical effects and cell-based bioassays with more specific effect observations. After single substance dose-response determination, bioactive substances were selected and two mixtures modeled. One mixture contained at least two active components for each bioassay that was able to detect single substance toxicity, thus allowing mixture effect elucidation within the bioassay panel to the greatest possible extent. A second mixture consisted of the same components but in environmentally relevant concentrations, thus aiming at reflecting naturally occurring circumstances and illustrating the impact of low-concentrated chemical mixtures on various organisms. Hence, observed mixture effects were compared against two mixture effect prediction models derived from additivity expectations, the model of concentration addition (CA) and independent action (IA). Most of the assays detected the mixture response of the active components as predicted even against a background of inactive contaminants. When none of the mixture components showed any activity by themselves then the mixture was also without effects. With one exception, the mixture effects observed using apical endpoints mainly appeared within the prediction window set by both applied models, whereas the specific cell- and organism-based endpoints resulted in mixture responses according to the model of CA. Concluding, most effect-based bioassays were able to quantitatively detect the predicted mixture effects, thus illustrating the importance of including mixture toxicity evaluation in risk assessment and further supporting the idea of combining chemical with bioanalytical monitoring tools.

**RP167 Suitability of growth rate in the marine amphipod *Parhyale hawaiensis* as an endpoint for chronic toxicity assessment**

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*Parhyale hawaiensis* is a marine amphipod with worldwide circum-tropical distribution. Recently, acute ecotoxicological procedures were standardized for these model organisms. However, it's potential for chronic toxicity assessment still unclear. The segmented body of *Parhyale* consists of the head, the thorax, and the abdomen. The specialization of *Parhyale* appendages offers interesting material to study the molecular and cellular biology, morphogenesis and growth, which is a standard endpoint in chronic tests. *P. hawaiensis* has become a model for evolutionary and developmental biology research, but no consistent information about growth rate was found in the literature. Thus, the aim of this study was: (i) obtain data on growth of *P. hawaiensis* during 100 days in a miniaturized system; (ii) determine the best time interval to perform chronic testing, based on growth rate data. Two independent experiments were performed. Each test consisted of 100 days, being 60 organisms studied in the first test and 72 organisms in the second. On the first day of observation, organisms at ages < 7 days were taken from the laboratory culture, photographed under a stereomicroscope and transferred to 12-well microplates containing 4 mL of salt water. Three times per week, water was partially renewed and organisms were fed. The organisms were monitored daily for molts and mortality. Every week a photo was taken to study the growth rate, more specifically in terms of cephalothorax length (mm). It was possible to observe a linearity of growth in both tests, growth rate global of 0.017 mm.day $^{-1}$ and 0.016 mm.day $^{-1}$ on tests 1 and 2, respectively. The measured mean lengths of cephalothorax were 1.300 mm and 3.122 mm, on days 0 and 100 respectively of test 1, and 1.215 mm and 2.782 mm, on days 0 and 100 respectively of test 2. Significant growth is observed from 21 days of observation for both tests (ANOVA on ranks, Dunn's ($p < 0.05$)). A decrease in the growth rate is observed from the day 70 for test 1 and 63 for test 2. Thus, to ensure the sensitivity of the endpoint, duration of the test should last at least 21 days and at most 63-70 days. More experiments are going to be conducted with the exposure of neonates to selected toxicants to verify if will effect on growth rate in the developed experimental conditions. *Acknowledgement:* CNPQ (CNPq-PVE Process: 400362/2014-7) for funding and PIBIC for undergrad fellowship. Amanda dos Santos and Gabriel R. Magalhães.

**RP168 The effects of sea lice chemotherapeutants on non-target Pacific prawns (*Pandalus platyceros*)**

*J. Bennett, C.J. Kennedy, Simon Fraser University / Department of Biological Sciences*

To counter the challenges from diseases, parasites, and fouling organisms that can affect the health of cultivated species, the aquaculture industry utilizes a variety of regulated chemicals loosely categorized as disinfectants, antifoulants, and medicines. A number of compounds have been used, or are currently being used, to treat sea lice infestations in salmon aquaculture. There exists a notable lack of information specific to the toxicity of these chemotherapeutants to non-target species found on the Pacific coast of North America where salmon aquaculture is common. Standard prawn culture techniques were used to raise larval, post-larval, and juvenile stages of spot prawn. Acute bioassays were performed for the determination of various toxicological parameters (LC25, LC50, EC25, EC50 with 95% confidence limits). In these tests with 4 life stages (newly hatched, Stage III, and Stage V postlarval stage, and adults), exposures for all chemicals were 1, 2, and 3 h in duration. Prawns were exposed to the formulations Salmosan®, Paramove®50, and SLICE®*via*water. Adults were examined in sublethal exposures for various alterations in physiology and behaviour. Data for 3 developmental stages (larval stages I, III, and V) of the spot prawn indicate that these early life stages are far more sensitive to all chemotherapeutants compared to adults. Salmosan®and SLICE®are more acutely toxic than Paramove®50, and there is some variability in sensitivity between developmental stages. Prawnsactively avoided SLICE®, Salmosan®, and Paramove®50 at various concentrations. Exposure of adults to these formulations resulted in increased oxygen consumption and several changes in locomotory and food-related behaviours. The data obtained from this project is required to ensure the proper and safe use, and appropriate regulation of these aquaculture chemicals in coastal ecosystems.

**RP169 Tools for Analyzing Concentration-Response Data: A Comparative Assessment for Supporting EPA Aquatic Life Water Quality Criteria Derivation**

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Derivation of Clean Water Act Section 304(a) Ambient Water Quality Criteria for the protection of aquatic life typically rely on empirical, laboratory-based aquatic toxicity values. Acute toxicity values are represented as $LC_{50}$ values. Chronic toxicity values were historically based on no-or-low observed effect concentrations (NOEC, LOEC) determined through hypothesis-based testing; however, recent aquatic life criteria documents have preferentially relied on chronic toxicity values determined as point estimates (e.g., $EC_{20}$), due to inherent limitations in hypothesis-based testing. For example, effect concentrations determined from hypothesis-based testing are heavily influenced by the concentrations tested, which limits consideration of concentration-response relationships. Therefore, the ability to generate, interpret, and select the most representative and reliable statistical fits from concentration-response (C-R) data is increasingly important for determining robust acute and chronic effect concentrations (i.e., $LC_X$, $EC_X$). EPA's Toxicity Response Analysis Program (TRAP) has routinely been used to support effects assessments and Aquatic Life Water Quality Criteria; however, alternative curve-fitting programs, including EPA's Benchmark Dose Software (BMDS) and R software, may provide additional models and guidance for fitting and interpreting C-R data. The goal of this research is to evaluate the use of TRAP, BMDS, and R for estimating acute and chronic effect concentrations across a range of pollutants, taxa, and effect levels. This assessment comparatively asses model output across curve-fitting programs and provides a framework for selecting and documenting appropriate C-R models to estimate effect concentrations and support the derivation of Aquatic Life Water Quality Criteria

**RP170 Toxicity effects of polyhalogenated carbazole compounds to a model organism the nematode *C. elegans***

*S.V. Sambukumar, K.A. Lenz, H. Ma, University of Wisconsin, Milwaukee / Zilber School of Public Health*

Polyhalogenated carbazoles (PHCZs) have been widely detected in river deposits and lake sediments across North America Great Lakes during the past two decades. The structural similarity of these PHCZs with halogenated dibenzofuran derivatives raises concerns about their potential dioxin-like toxicity. Here we investigated toxicity of five PHCZs (carbazole(CZ); 2,7-dibromocarbazole (27-DBCZ); 3,6-dibromocarbzaole (36-DBCZ); 3,6-dichlorocarbazole (36-DCCZ); 1,3,6,8-tetrabromocarbazole (1368-TBCZ)) to a model organism the nematode *C. elegans.* 2,3,7,8-TCDD was also included in the study for comparisons. Apical toxicity endpoints included lethality, reproduction, and lifespan. Germline toxicity was evaluated using a *xol-1::GFP* transgenic strain. Impact on gene expression at sublethal concentrations was examined for groups of genes involved in heat-shock response (*hsp 16.1, hsp 16.2, hsp 16.48, hsp 70*), oxidative stress (*sod1~5, ctl1~3, daf-2, daf-16, gcs-1, gst-4, skn-1*), xenobiotic metabolism (*cyp-35a1~5*) and development (*vit-2, vit-5~6*). Acute toxicity (24-h LC50) showed the following trend: 27-DBCZ(0.15µM) > 36-DCCZ(0.44 µM) > 36-DBCZ(2.05 µM) ≈ 2378-TCDD(3.2 µM) > 1368-TBCZ(10.3 µM) ≈ CZ(12.1 µM), with 27-DBCZ being the most toxic and CZ the least toxic. Impact on worm reproduction occurred at concentrations one order of magnitude lower than LC50s and was concentration dependent. Impact on lifespan showed similar patterns as reproduction, with 27-DBCZ, 36-DCCZ, and 36-DBCZ showing greater impact than others. All five PHCZs and TCDD showed germline toxicity as indicated by "green eggs". Impact on gene expression depended on the chemicals as well as the genes examined. Significant upregulation (fold-change of 4~6) of *hsp* genes was only observed for 27-DBCZ. Downregulation (fold-change of 3~4) of *vit* genes was only seen for CZ. Among all the oxidative stress related genes examined, only *sod-3* showed slight downregulation for 27-DBC, 36-DBC, 36-DCC, and 1368-TBC (fold-change of 2~4), and *sod-5* showed upregulation for CZ and TCDD (fold-change of 4~5). For xenobiotic metabolism related genes, 1368-TBC induced significant upregulation in all five *cyp-35a* genes (fold-change of 14~466). Our findings suggest these PHCZs induce systemic toxicity effects in the worm, and the toxicity is dependent on the chemical. Impact on *cyp35a* genes warrants further investigations as it may shed light on understanding the mechanisms of toxicity for these PHCZs as compared to TCDD.

# Assessing the Risk of Complex Mixtures: Advances and Challenges

### PC001 Substance mapping: A first step towards developing a better assessment strategy for UVCBs

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Risk assessment of complex substances (e.g., multi-constituent substances (MCs), and substances of Unknown or Variable Composition, Complex Reaction Products, and Biological Materials (UVCBs)) presents numerous challenges. International regulatory programs (specifically REACH, Canada's Chemicals Management Plan, and USEPA's PMN process) have highlighted the complexities of characterizing fate and exposure, risk assessment, and registration of these types of substances. A new HESI Project Committee was started in early 2018, with the goal of developing a tiered approach to assess ecological risks of MCs / UVCBs, and meet regulatory needs. The Committee determined that mapping the vast and complex universe of UVCB substances by chemical and/or functional classes should be the foundation of all work performed on this project, as a key to better understand the nature of these substances and their sector of use, and provide insight on which of them present the largest regulatory challenges. This exercise will also help identify UVCBs of most concern to the environment and human health. The classification of UVCBs according to criteria such as chemistry similarities and use patterns could ultimately help the development of strategies and streamline the risk assessment of these substances. Examples of UVCB mapping by participating regulatory agencies will be presented and some of the most common categories of UVCBs will be highlighted along with the technical challenges associated with risk assessment of these classes. Depending on the identified classes of UVCBs, it may be possible to apply many of the existing analytical and assessment methods to characterize UVCBs and evaluate their hazard potential. An important starting point, however, is to have the most accurate information to identify and characterize the MCs and UVCBs to ensure that their chemical classification is accurate. [*The views of the authors of this presentation are those of the authors and do not represent the views of their respective organizations*]

### PC002 Considerations for Measuring Exposure to Chemical Mixtures

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Exposure to chemical mixtures contributes to human disease risk. Analytical capability is continuing to increase, but the toxicities and environmental occurrences of many chemicals remain understudied. If a commercial chemical standard does not exist for a given chemical, then little to no quantitative data likely exists for that chemical. This presentation will provide the highlights from a chapter in *Chemical Mixtures and Combined Chemical and Nonchemical Stressors*, a book that was published in winter of 2018. This presentation will discuss exposures to organic chemical mixtures, necessary considerations for studying those exposures, and research needs to continue to advance the field of mixtures exposure science. When planning or reviewing studies that focus on exposure to chemical mixtures, important considerations include: spatial orientation of sampling, temporality of sampling, bioavailability of measured chemicals, measuring enough of the appropriate chemicals, potential for chemical transformations, and mixture effects. This presentation will also use case studies to illustrate how these considerations can be applied when designing a study or interpreting results from a study. The case studies focus on assessing the potential for emissions from natural gas extraction (NGE) to affect polycyclic aromatic hydrocarbon (PAH) exposures of people a) eating food crops grown near active NGE wells, and b) living or working near active NGE wells. Importantly, relatively little is known about how exposure to chemical mixtures differs from exposure to individual chemicals. Most toxicity studies are performed using individual chemicals and nearly all exposures involve chemical mixtures. Characterizing the toxicity of chemical mixtures should therefore be a priority for the scientific community.

### PC003 Bioconcentration factors of constituents of pine oil in fish determined in an in-vivo benchmarked dietary exposure study

*M. MacLeod, C.L. Chen, Stockholm University / Department of Environmental Science and Analytical Chemistry ACES*

Essential oils are fragrance materials that are registered as natural complex substances (NCS) under the European REACH legislation. One of the categories of information required in a REACH registration is information about the potential for bioaccumulation of NCS by fish. Determining the bioconcentration factor (BCF) of essential oils cannot be readily accomplished using a standard flow-through uptake/depuration experiment. Previously, we demonstrated that a single dietary exposure coupled to the benchmarking technique could be applied to an artificial mixture for measuring the in vivo BCF. Here, we report an application of our proposed BCF-determination methodology on a real essential oil – pine oil. Fish (rainbow trout) were dosed with a mixture of the pine oil and a suite of benchmark chemicals via a single dietary exposure. The depuration rate constants ($k_T$) in the fish soma (without GIT) for the key pine oil constituents are 0.134 $d^{-1}$ (β-Caryophyllene, BCP) – 1.41 $d^{-1}$ (BAc) and they were 0.0.0799 $d^{-1}$ (HCB) – 0.517 $d^{-1}$ (DiCB) for the reference chemicals. The test compounds depurated faster from the soma than the GIT, making estimated whole body depuration slower (conservative) compared to the soma only. HCB was the chemical most resistant to depuration among all the test compounds. Benchmarking to HCB reduced the standard error of measured $k_{T\text{-}BM}$ from the soma for most of the chemicals, with $k_{T\text{-}BM}$ ranging from 0.001 $d^{-1}$ (PCB52) to 2.98 $d^{-1}$ (BAc). The apparent BCF ($BCF_A$) values in soma for the key components in pine oil and the reference chemicals were in the range of 98.2 L $kg^{-1}$ (BAc) – 1030 L $kg^{-1}$ (BCP) and 267 L $kg^{-1}$ (DiCB) – 1730 L $kg^{-1}$ (HCB), respectively; while for the benchmarked BCF ($BCF_{BM}$) in soma, they are 46.3 L $kg^{-1}$ (BAc) – 2050 L $kg^{-1}$ (BCP), and 208 L $kg^{-1}$ (DiCB) – 197000 L $kg^{-1}$ (PCB52) repectively. We conclude that a single dietary expourse coupled with the benchmarking technique is a feasible experimental approach for measuring the BCF of NCS in fish.

### PC004 Threshold of Toxicological Concern risk assessment for a mixture of chlorinated aromatic sulfonic acid chemicals through drinking water exposure

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Threshold of Toxicological Concern (TTC) risk assessments are used by some regulatory agencies as tools for determining levels of exposure to chemicals of undetermined toxicity below which there is minimal concern and for prioritizing additional evaluation where TTCs are exceeded. Effluent from a treatment system for groundwater at a hazardous waste landfill contains a mixture of polar chlorinated aromatic sulfonic acid compounds that are byproducts of historical DDT production and which are not removed by treatments systems designed to remove other contaminants. A contract laboratory utilized liquid chromatography and electrospray ionization tandem mass spectrometry to tentatively identify and estimate the concentrations of the constituent compounds in the undiluted effluent. Since chemical specific toxicological information is unavailable, a TTC risk assessment was performed. TTC classification was performed using the software, Toxtree. In addition, other publically available in silico tools were utilized to support the TTC classification. Drinking water equivalent levels (DWELs) for the TTCs were calculated using California Office of Environmental Health Hazard Assessment (OEHHA) age-specific drinking water ingestion rates. TTC DWELs can be used by the publically owned treatment works that ultimately receives the effluent to determine needed analytical reporting limits as well determine the need for additional risk evaluation or management. The TTC risk assessment will be presented.

**PC005 Application of two frameworks for estimating mixture toxicity of fish contaminants**

*N. Urban, J.A. Perlinger, Michigan Technological University / Civil and Environmental Engineering; E. Shaw, Michigan Technological University*

Historically, fish consumption advisories (FCAs) have been based on the most sensitive endpoint (lowest reference dose) for single contaminants. Given that there are always multiple contaminants present in fish, that some contaminants may share common health effect end points, and that many contaminants influence the uptake or clearance or degradation of other contaminants, this single contaminant approach may inadequately assess the risk of fish consumption. Given that synergistic effects among contaminants are seldom observed and that we do not yet know many of the contaminant interactions that do occur, it has been argued that chemical additivity (CA) is an appropriate model for estimating total risk. In this study, we apply the CA model in two ways to estimate cumulative toxicity in fish to answer three questions: 1) What are the major contributors to the total toxicity of dioxin-like compounds (DLC) in fish? 2) What are the relative contributions of the two dominant fish contaminants (mercury, PCBs) to cumulative toxicity? And 3) What is the variability in cumulative toxicity relative to that due to single contaminants? We utilize two data sets for this analysis: Michigan's Fish Contaminant Monitoring Program and EPA's National Fish Tissue Survey. Within the Michigan data set, two PCB congeners accounted for the majority of the DLC; in all fish, PCB contributions to the total toxic equivalents exceeded that of dioxins and furans. Mercury and PCBs were observed to accumulate in different types of lakes; mercury was dominant in small, inland lakes and PCBs were dominant in larger lakes including the Great Lakes. As a result, the variance in total toxicity was less than the variance for the individual contaminants.

**PC006 ITRC TPH Risk Assessment Guidance Document**

*A. Goldberg Day, ARCADIS*

Background. Total petroleum hydrocarbons (TPH) mixtures are not always evaluated in human health or ecological risk assessments. Instead a somewhat arbitrary 100 parts per million is often applied as a cleanup goal. To address the lack of TPH criteria, TPH is often evaluated using surrogate chemicals such as benzene, naphthalene, and ethylbenzene. To address the lack of TPH risk assessment guidance, the Interstate Technology Regulatory Council (ITRC) will be publishing a TPH Risk Assessment guidance document, due to be released in November 2018. ITRC Guidance Document. The objective of this guidance is to build on previously published research to assist users in evaluating potential risks and risk-based corrective action assessments at petroleum hydrocarbon spill and release sites. In general, this guidance will present the current science and understanding of TPH risk characterization. The document will present why TPH risk characterization is important and provide a resource to guide users in making technically defensible assessments at TPH impacted sites to protect human and environmental health. The document will also describe the unique issues that should be addressed when characterizing TPH risks. Applicability and limitations of this will also be presented. Presentation. This presentation will discuss the methods for performing both human health and ecological risk assessment. This includes providing information the physico-chemical properties that determine the behavior of petroleum hydrocarbons in the environment and the various analytical methods available. Sampling strategies for both human health and ecological evaluations will be included. The ITRC TPH document will also identify the tool gaps for evaluating human health and ecological risk for TPH. Where appropriate, advice will be provided on how to address these gaps so that risk-based conclusions can be as complete as possible and the resulting risk information effective in terms of supporting risk management decisions. A demonstration of the on-line tool will also be included.

**PC007 Developing a strategy to improve the environmental risk assessment of substances of unknown and variable composition (MCS/ UVCB)**

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The complexity of Multi-Constituent Substances (MCS) and Substances of Unknown or Variable Composition, Complex Reaction Products, and Biological Materials (UVCBs) pose a unique challenge to regulators and to product registrants having to characterize their fate, exposure, hazard, and potential risks to human health and the environment. International regulatory frameworks (specifically REACH, Canada's DSL Categorization and Chemicals Management Plan, and USEPA's PMN process) have highlighted the difficulties of registering these products. A new HESI Project Committee was started in early 2018, with the goal of developing a tiered approach to assess ecological risks of MCS / UVCB to address scientific and regulatory needs. Through the engagement of stakeholders facing challenges associated with these substances, this Committee will foster the exchange of knowledge about MCS / UVCB, and develop and optimize a cross-sectorial approach for the classification, identification, characterization and assessment of these substances. In first step, the Committee focused on the mapping of the vast and complex universe of UVCB substances to provide a foundation for subsequent work, and better understand the nature of these substances and their sector(s) of use. It will also provide insight on which groups of substances present the largest regulatory challenges and where strategic approaches for assessment would be most valuable. The outcome of the mapping exercise will also inform how to apply existing analytical assessment methods such as read-across, QSAR and trend analysis to characterize UVCBs and evaluate their potential hazard. It may also allow for the identification of analytical gaps and inform the development of new UVCB identification and characterization methods. Finally, part of this effort will be devoted to the review and evaluation of existing sector-specific MCS / UVCB assessment tools with an aim towards making them more widely applicable to other substances and/or to develop new assessment tools and methods that apply to other substance groups. Ultimately, this project will lead to the development of best practices to appropriately evaluate the environmental fate, and assess the ecotoxicity of MCSs / UVCBs.

## Endocrine Disruption in Invertebrates: Historical Perspectives, New Developments and Key Research Needs

**PC008 Endocrine disrupting effects of organic ultraviolet-filters on *Daphnia magna* molting and development**

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Organic Ultraviolet-filters (UV-Fs) are emerging contaminants of concern and ubiquitously found in the aquatic environment. Many organic UV-Fs are endocrine disrupting compounds (EDCs) in vertebrates. However, only a handful of studies have assessed their potential to cause endocrine disruption in invertebrate species. The process of molting, or the shedding of the exoskeleton in arthropods, has been a suggested target of these compounds. Molting is necessary for growth and development and is regulated by an arthropod-specific endocrine system, the ecdysteroid pathway. Alterations of this process by EDCs can result in improper development, reduced growth, or death, resulting in potentially significant implications for organism and population health. In this study we investigate the hormonal activity of organic UV-Fs in a crustacean. *Daphnia magna* are chronically exposed to three common organic UV-Fs: 4-methylbenzylidene (4MB3), octylmethoxycinnamate (OMC), and benzophenone-3 (BP3) and assessed for alterations in normal molting and development. Additionally, changes in expression of the ecdysteroid regulated genes *EcRA, EcRB, HR3,* and *E75* are evaluated upon acute exposure to 4MBC, OMC, and BP3. This study is the first to assess the ability of UV-Fs to act as endocrine disruptors of ecdysteroid regulated processes in a crustacean.

**PC009 Bioaccumulation of 4-nonylphenol in blue crab, *Callinectes sapidus*, juveniles does not affect growth but exponentially increases hsp90 expression**

*S. Chiasson, Loyola University / EEB; C.M. Taylor, Tulane University / Ecology & Evolutionary Biology*

Endocrine disrupting chemicals (EDCs) enter the environment from anthropogenic sources, and some of these chemicals have estrogen mimicking properties. In our previous study 4-nonylphenol (NP) was detected in post-larval blue crabs, *Callinectes sapidus* at concentrations that exceeded the No Observed Effect Concentration for aquatic invertebrates. The blue crab, *Callinectes sapidus*, is an ecologically and economically important invertebrate that lives in coastal estuaries. In this study, juvenile blue crabs were exposed to seven sub-lethal (0 – 200 ppm) concentrations of NP, and accumulation was measured in crabs after a 24-hour exposure using GC/MS. Also, mRNA expression of genes encoding heat shock protein 90 (*hsp90*) and vitellogenin (*vtg*) were measured in crab hepatopancreas tissue. Crabs were found to linearly accumulate NP, whereas expression of *hsp90* in the hepatopancreas increased exponentially with NP concentration. To test for effects on juvenile blue crab growth at environmentally relevant concentrations, crabs were exposed to NP and monitored in tanks for one molt cycle. Expression of *vtg* was not affected by exposure to NP, and no significant differences were detected in growth or intermolt duration. This study shows that bioaccumulation of NP does not appear to affect growth at the measured sub-lethal concentrations whereas *hsp90* expression increased exponentially, linking NP exposure to two different endpoints.

**PC010 Atypical Length Responses in Mysid Chronic Testing – Are They Relevant to Risk Assessment**

*L. Sayers, R.C. Biever, Smithers Viscient / Department of Ecotoxicology*

Invertebrate life cycle exposures are a key element to the requirements for many regulatory bodies for risk assessment. Mysid shrimp (*Americamysis bahia*), a marine invertebrate, are a relevant species for this evaluation as they are potential indicators of effects test chemicals on aquatic invertebrates. In the United States, several documents exist to provide guidance on developing data for the chronic exposure of chemicals to mysid shrimp (USEPA, ASTM) however European requirements (i.e. REACH) have no specific requirements for marine species. Thirty mysid chronic studies were evaluated, seven of which were conducted utilizing a co-solvent. These exposures were conducted between 2013 and 2017. In review of the length data associated with these studies, an interesting trend appears. For length in particular, 40% of the data sets contain results where all treatment values are less than the control for male mysid and 30% of all treatment values are less than the control for female mysid. This pattern might be expected for a traditional ecotoxicology dose response however, additional data sets occur where all lengths for treated male and female mysid are all greater than the controls (10% and 17% of the data sets, respectively). The obvious prevalence of data sets where results for the treatments are, in their entirety, either greater than the control or less than the control speaks to a trend that may need to be considered in the interpretation of a toxicant related effect (either enhancement or suppression). This talk will explore these data sets with an eye towards biological relevance in the risk assessment setting and current guidance on biological relevance (EFSA, 2017). In the case of either complete suppression or enhancement, it is reasonable to conclude that there is some cause for this response even if the response is not significantly different from the control or from historical control values. However, if this response does not have an effect on a population level (i.e. reproduction is not impacted) or a corroborating effect in the weight endpoint, these effects may not be relevant in the risk assessment setting.

**PC011 Intersexuality in Aquatic Invertebrates: Prevalence, Causes, Toxicological Mechanisms and Research Directions**

*T. Grilo, R. Rosa, MARE – Marine and Environmental Sciences Centre*

This study intends to assemble information on intersexuality in aquatic invertebrates, from freshwater to estuarine andmarine environments. Intersex is a condition whereby an individual of a gonochorist (separate sexes) species has oocytes or distinct stages of spermatogonia, at varying degrees of development, within the normal gonad of the opposite gender (i.e. spermatocytes in the ovary or oocytes in the testis), often involving alterations in the gonadal structure, reproductive tract or external genitalia. By the end of 2016 we found approximately 340 records of aquatic invertebrate species evidencing signs of intersexuality (or imposex), all comprised within the Phyla Mollusca and Arthropoda. Gastropod molluscs are by far the group with more examples documented (256 species), followed by crustaceans, i.e., decapods, copepods and amphipods. To our knowledge no further cases of intersexuality were known concerning other invertebrate taxa. Despite some reports suggesting that a baseline level of intersexuality may occur naturally in some populations, the causes are multifaceted and mostly linked with environmental contamination by estrogenic and organotin endocrine disrupting chemicals (EDCs), parasitism, and genetic/environmental sex determination abnormalities. A more comprehensive discussion about the origin of intersexuality, prevalence and causes, knowledge gaps and future research directions in the light of new omics scientific advances (genomics, proteomics and transcriptomics) is also provided. The lack of studies linking molecular responses of invertebrate intersex individuals to multiple stressors represents a true challenge to be further investigated in the future.

## Ecological, Human Health, Economic and Social Impacts of Wildland Fires

### PC012 Setting the Stage: Ecological, Human Health, Economic and Social Impacts of Wildland Fires

*C. Baghdikian, USEPA / Office of Research and Development / National Health and Environmental Effects Research Laboratory*

Fire is vital to maintaining ecosystem functioning, however there are tradeoffs. Increasing wildland fires pose direct risks to human health and ecosystem services--air quality, clean water. More people are living in high risk areas, often referred to as the Wildland-Urban Interface (WUI), resulting in increasing risks from smoke exposure, and higher risk to life and property. Further, fire-prone ecosystems, forests in most cases, are sources of our national water supply—wildland fires can result in water quality degradation from sediment and nutrient transport, as well as decreased storage capacity in damaged watersheds. This presentation will set the stage for the rest of the session, where you will hear from experts across the landscape of wildland fire research.

### PC013 The High Cost of Burning: Economic Costs of Wildland Fires and Smoke in the US

*B. Hubbell, USEPA / Office of Research and Development; D. Butry, NIST*

The prevalence and severity of wildfires has been increasing over the past decade due to both past fire management practices and changes in climate. At the same time, the wildland-urban interface has been increasing, which potentially increases the economic risks associated with wildfires. Economic costs arise directly from activities to control fires and protect populations (e.g. evacuations), the burning of structures and property, adverse impacts on watersheds and ecosystems, and also through property, environmental, and health impacts from exposures to smoke from wildland fires. These costs can occur in communities far distant from the location of the fires. Costs are also associated with increased fire management activities, including increased numbers of fire fighters and related costs, and increased costs to manage fuel loads and carry out prescribed and controlled burns. In addition, costs to insure at-risk property can be significant. This presentation will provide an overview of research on the economic costs from wildland fires, with a focus on costs associated with smoke exposures. Direct costs of wildfire suppression have been fairly well characterized, with estimates of $2 to $3 billion per year. Estimates of total costs of wildfires including both costs of suppression and value of wildfire related impacts (both beneficial and harmful) are more variable, but a recent estimate indicates total annualized net economic costs of $71.1 to $347.8 billion. The costs associated with smoke from wildland fires is a growing area of research. These smoke-related costs have the potential to be very substantial, given the potential for widespread exposures to wildfire smoke in heavily populated areas coupled with the strong evidence linking smoke and a range of health effects that includes symptoms such as eye irritation, sore throat, wheezing, and coughing worsening of respiratory diseases like asthma and chronic obstructive pulmonary disease, bronchitis, pneumonia, cardiovascular events, increased rates of cardiorespiratory emergency department (ED) visits, adverse birth outcomes, hospitalizations, and premature death. A recent study estimated that the annual economic burden of smoke related health effects over the period from 2008 to 2012 was nearly $100 billion. Additional costs related to smoke exposures, including impacts on livestock, structures, and ecosystems, have not been well characterized.

### PC014 Ecological Effects of Wildland Fire: Tradeoffs for Delivery of Ecosystem Services

*P. Beedlow, W.S. Fisher, USEPA / Office of Research and Development / National Health and Environmental Effects Research Laboratory*

Fire is a vital process for sustained ecosystem functioning, providing habitat vitality and renewal through recolonization of new populations and succession of fire-tolerant species. Twentieth century fire management practices have shown that removal of fire from fire-adapted ecosystems leads to catastrophic unintended fires, which are detrimental to both human health and ecosystem services. Because more people are living in high fire-risk areas, there are increased hazards for life and property. Moreover, fire-prone ecosystems, forests in most cases, are major sources of our national water supply, and catastrophic wildland fires reduce canopy, ground cover and soil cohesion, which decreases water storage capacity and can lead to water quality degradation from sediment, nutrients and toxic compounds eroding into waterbodies. Eliminating wildland fires is neither possible nor desirable, but there are ways to reduce risks to water resources and delivery of ecosystem services. For example, areas vulnerable to wildfire can be targeted for management actions such as fuel reduction and prescribed burning. To better manage the effects of natural, accidental and prescribed fires will require improved information and technology for predicting fires (spatial and temporal) and for gauging risks, as well as tools for education, communication and community planning.

### PC015 Human Health Impacts of Wildland Fire and Smoke

*W.E. Cascio, USEPA / NHEERL*

Wildland fire and smoke from these fires can impact public health. The annual acreage burned in the U.S. from wildfires has risen steadily since 1985, and the fire season has lengthened. Wildland fires impair air quality by producing massive quantities of particulate air pollutants and ozone precursors. Together particles and ozone exposures increase the risk of premature death and acute and chronic cardiovascular and respiratory morbidity among vulnerable individuals. Future wildfires are predicted to be larger, more severe and more frequent in some regions of the U.S and will contribute to an even greater proportion of the ambient air pollution, the disease burden and healthcare costs. While the projected magnitude of the public health impact of a changing environment on wildfire events is uncertain, it is clear that the proportion of the U.S. population vulnerable to the adverse health effects of wildland fire and its smoke is increasing. An aging population with chronic respiratory diseases and increasing obesity and diabetes that heralds more cardiovascular disease will increase the vulnerability of the population to the adverse effects of wildfire smoke and associated stressors. Additionally, physiological changes attendant to aging decrease the capacity of aged-adults to tolerate wildfire smoke, heat, humidity, evacuation and recovery. Expansion of our cities into the wildland-urban interface is also placing a greater proportion of the population in closer proximity to wildland fire emissions with its associated health risks. The public health community has an opportunity to contribute to the broader national effort to mitigate wildland fire risk by working closely with the healthcare community to facilitate adaptive responses to wildfires. Adaptation will increase the resilience of individuals and their communities and is anticipated to help mitigate the adverse health effects of wildland fire. *This abstract does not reflect USEPA policy.*

### PC016 Psychological and Human Behavioral Responses to Wildland Fire

*M. Brunson, Utah State University / Environment and Society*

Humans have both managed and feared fire for millennia. Public officials and society as a whole are generally aware that wildland fires are increasing in frequency, size, and cost across North America. Yet efforts to reduce the risk of catastrophic wildfire fall short of what is needed at both individual and community levels. At the individual scale, human perceptions of wildfire risk and risk-reduction strategies are influenced by a variety of factors including: resource availability, perceived self-efficacy, previous experience with natural hazards, perceptions of the risk-reduction strategy, generalized beliefs about nature and the society-environment relationship, generalized beliefs about hazards and risk, and local norms. Risk-reduction efforts often have undesirable side effects that may be more immediate than the hazard they're designed to reduce. At the community scale, economic and demographic factors as well as socio-political norms influence a community's resilience to wildfire as well as its capacity to undertake risk-reduction efforts. Understanding how these various factors interact, both within and across scales, can help policy makers craft more effective programs to help people and ecosystems recover after wildfire events as well as reduce the risk of future wildfires.

## Improving Approaches to Assess Risks to Threatened and Endangered Species from Chemical Exposure

### PC017 Use of USEPA Regulatory Models in a Refined Malathion Endangered Species Aquatic Exposure Assessment

*M.F. Winchell, H. Rathjens, Stone Environmental, Inc. / Environmental Systems Modeling; L. Padilla, Stone Environmental, Inc.; P. Whatling, FMC Corporation*

Recent Biological Evaluations and Biological Opinions concerning the effects of currently registered organophosphate insecticides on threatened and endangered species have adopted regulatory aquatic exposure models and scenarios to represent a wide range of species and habitat characteristics. The application and parameterization of these models to date has been largely at a screening level in terms of the conservativeness of assumptions and the spatial specificity of the exposure model inputs and predictions. The best nationally available spatial datasets provide the information necessary to improve the parameterization of regulatory aquatic exposure models such as the PRZM landscape and VVWM receiving water models, and their resulting exposure estimates. To evaluate the practical use of these models and datasets in a national scale endangered species assessment, 100 different species across a range of taxonomic families, occupying static and flowing habitat, were assessed. The static water modeling approach focused on independently evaluating yearly realizations of crop data to represent multiple cropping configurations surrounding small static water bodies. The proximity of crops to water refined the drift exposure and the distribution of soil types and weather refined the runoff potential. The flowing water modeling approach took advantage of the NHDPlus catchment network to determine contributing watershed areas for each distinct catchment within a species range. The NHDPlus network and associated flow length and flow velocity attributes determined the lag-times of pesticide loads contributing from upstream catchments, and determined the duration of chemical degradation. The flowing water modeling also considered multiple independent years of crop data to generate an ensemble of cropped area realizations in each NHDPlus catchment and upstream watershed. In both the static and flowing habitat modeling, historical pesticide use data was used to estimate the fraction of catchments receiving malathion applications for each crop group, which was varied for each of the five crop years modeled. This exposure modeling approach resulted in species-specific EEC distributions that could be further filtered according to habitat characteristics (flow rate, water body size), grouped according to contributing watershed characteristics, and mapped explicitly to locate regions within a species range that are likely to experience exposure levels of potential concern.

### PC018 Species monitoring with environmental DNA (eDNA): Sampling design considerations

*R.A. Erickson,C. Merkes, USGS / Upper Midwest Environmental Sciences Center; E.L. Mize, USFWS / Whitney Genetics Lab*

Natural resource managers require knowledge of species distributions in order to manage the species. Within ecotoxicology, this is especially important so that exposure can be estimated. However, rare, cryptic, or otherwise hard to detect species can be difficult to observe. This is especially true for threatened or endangered species and newly arriving invasive species. One tool to detect these species is environmental DNA (eDNA). eDNA-based methods sample water, air, soil, or other locations in the "environment" of the species of interest. These samples are then analyzed using molecular tools such as quantitative polymerase chain reaction (qPCR) or sequencing. An important consideration of eDNA-based sampling methods is imperfect detection. When sampling a location, a species may not be present all of the time. Within a site, a sample (e.g., water collection) may not contain eDNA by chance alone. Last, the molecular methods may have imperfect detection. Occurrence models are one method for accounting for these different levels of imperfect detection. These models can be used to estimate the probabilities of site occurrence, sample capture, and detection. Through the use of probability calculations and simulation, we compared different sample designs, with a focus on rare species (i.e., those with low detection and capture probabilities). We found detecting eDNA in ≥1 sample at a site required ≤15 samples per site for common species, but detecting eDNA when looking for rare species required 45-90 samples per site. Our occurrence model recovered known parameters unless capture and detection probabilities were < 0.2 where >100 samples per site and ≥8 molecular replicates were required. Our findings illustrate the importance of sample size and molecular replication for eDNA-based work.

### PC019 Beyond Population Modeling for Endangered Species Risk Assessment

*D. Preziosi, B. Sackmann, E. Mendelsohn, R.A. Pastorok, Integral Consulting, Inc.*

Population modeling is an essential tool for assessing pesticide risks to endangered species. But is modeling at the population level sufficient to protect rare species? We evaluate the role of ecosystem modeling in endangered species risk assessments, particularly with respect to potential indirect effects mediated via species interactions (e.g., trophic pathways). Inter-species interactions within the biological community in most ecosystem models focus on predator-prey (or grazer-resource) interactions and intra- and inter-species competition. We present examples of trophic cascades and address how ecosystem modeling can support endangered species risk assessments. Ecosystem modeling allows consideration of not only indirect effects but also other physical stressors such as habitat temperature modification associated with climate change or physical removal of habitat during human development. In certain cases, other interactions are added to such models; for example symbiotic relationships for coral-algal species and parasitoid-host relationships for agricultural settings where biological control is part of an integrated pest management system. We explore challenges in ecosystem modeling, including some specific to endangered species and also common to population modeling. With ecosystem models, the challenges are multiplied by the complexity of the food web being modeled. A framework for use of ecological models for endangered species should combine multi-modeling approaches; classification of endangered species by life history characteristics and vulnerability; and methods to estimate biological and ecological parameters of rare species from surrogate species data.

### PC020 Croplife America Ecological Risk Assessment Framework for Endangered Species Assessments

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Ensuring that populations of threatened and endangered species in the United States remain viable is an important factor in enhancing our environment and maintaining biodiversity. While habitat loss is often the primary risk to listed species, endangered species risk assessments (ESRA) can identify cases in which exposure to pesticides could potentially increase risk, in some situations. Pesticide registration and re-registration under Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) administered by the Environmental Protection Agency (EPA) constitutes a federal action and thus is subject to review under Section 7 of the Endangered Species Act (ESA), administered by the Fish and Wildlife Service (FWS) and National Marine Fisheries Services (NMFS) (collectively called the Services). In the 2013 report entitled “Assessing Risks to Endangered and Threatened Species from Pesticides”, the National Academy of Sciences (NAS) provided guidance for conducting endangered species risk assessments (ESRA) under FIFRA with direct consideration of the ESA Section 7 requirement. The NAS panel recommended the use of refined approaches (e.g., probabilistic methods, population modeling) when conducting assessments. In response to the NAS panel report, EPA, the Services, and USDA released a summary of their implementation plan (often called the ‘Interagency Interim Approach’) that presented a common approach to ESRA. The Interagency Interim Approach was applied in the development of the recent organophosphate (OP) (malathion, chlorpyrifos, and diazinon) biological evaluations (BEs). Unfortunately, these BEs illustrated that the Interagency Interim Approach is inefficient (e.g., failing to remove many species that are clearly not at risk), does not follow a tiered approach, is not scientifically defensible, and will result in unacceptable delays in regulatory decisions and require large increases in Agency resources, all with uncertain benefit to listed species and their critical habitats. This presentation presents a framework for conducting efficient ESRA using a conservative but scientifically defensible and rational approach adapted from the National Academy of Science panel report recommendations, the Overview of the Ecological Risk Assessment Process in the Office of Pesticide Programs, the 1998 EPA Risk Assessment Framework, and lessons learned from the OP case studies.

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